CELL WALL STRUCTURE

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Cell Envelope

The cell envelope may be defined as the cell membrane and cell wall plus an outer membrane if one is present.

The cell wall consists of the peptidoglycan layer and attached structures.

Cell Wall

- They are composed of unique components found nowhere else in nature.
  - **Bacteria - Peptidoglycan**
    - Exceptions:
      - **Mycoplasmas – Lack cell walls**
  - **Archaea – no peptidoglycan**

- The cell wall of bacteria is **an essential structure for viability**.
  - **Protects the cell** (**protoplast**)from mechanical damage and from osmotic rupture or **lysis**
- They provide **ligands for adherence**.
- They cause symptoms of disease in animals.
- Receptor sites for drugs or viruses.
  - They are one of the most important sites for attack by antibiotics.
- They provide immunological variation among strains of bacteria.

Peptidoglycan

A single bag-shaped, highly cross-linked macromolecule that surrounds the bacterial cell membrane and provides rigidity.

Peptidoglycan consists of:

1. Glycan (polysaccharide) backbone consisting of
   A. N-acetyl muramic acid (Mur)
   B. N-acetyl glucosamine (Gln)
2. Peptide side chains containing
   A. D- and L- amino acids
   B. Diaminopimelic acid (in some cases)
   C. The side chains are cross-linked by peptide bridges which vary in structure among bacterial species.

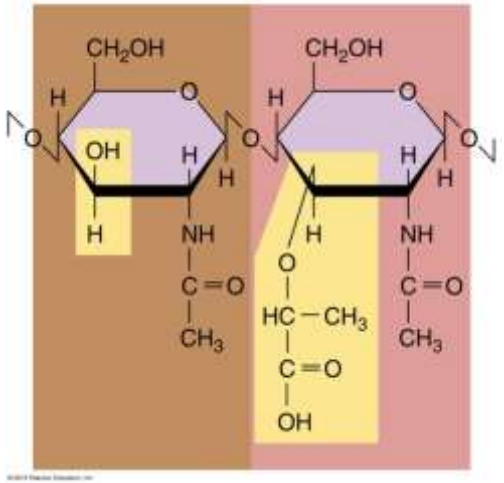
Cell Wall - Peptidoglycan

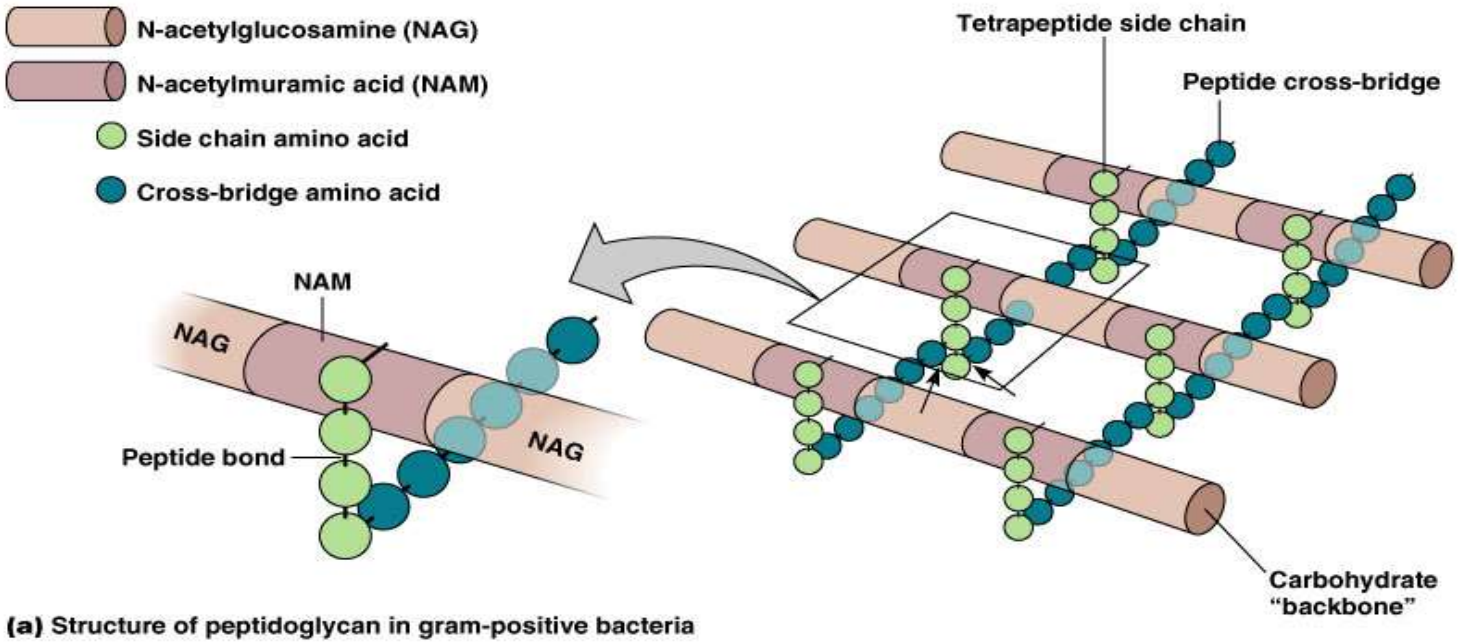
- Polymer of disaccharide

$(NAG\text{-}NAM)_n$

- Linked by polypeptides

N-acetylglucosamine (NAG) - N-acetylmuramic acid (NAM)



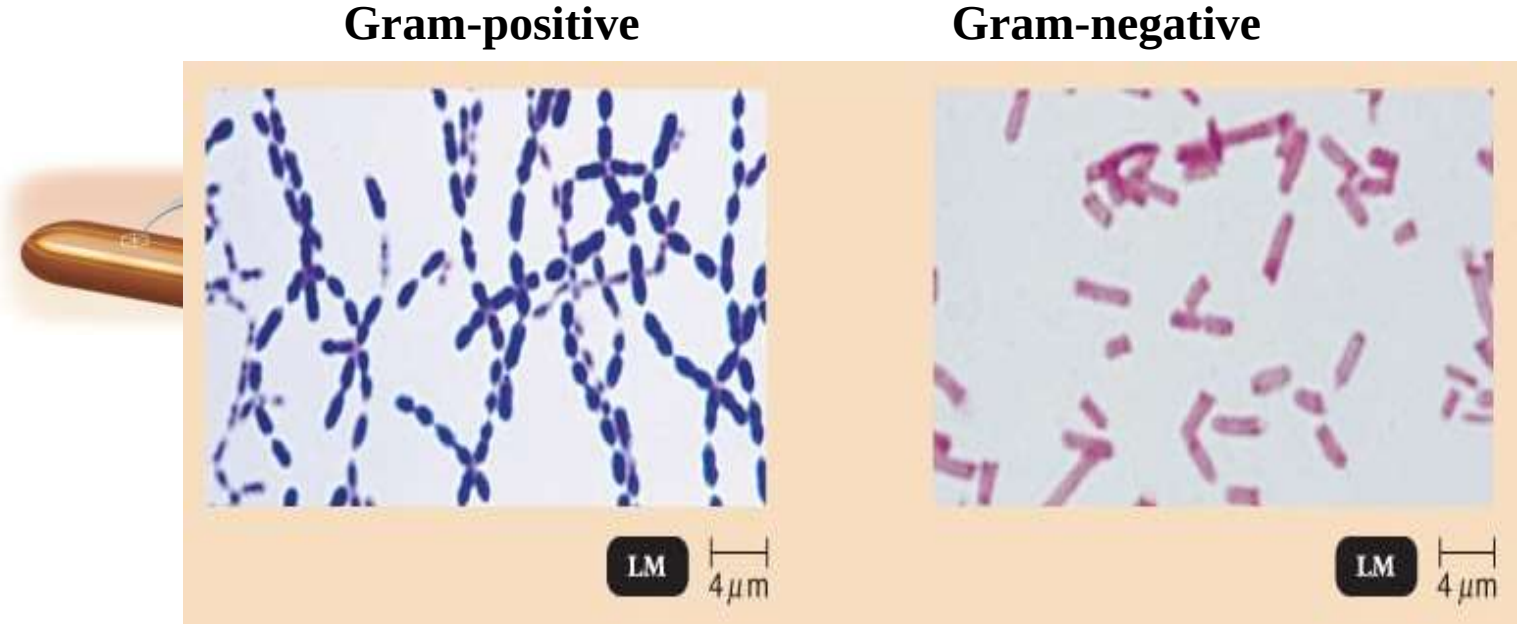


(a) Structure of peptidoglycan in gram-positive bacteria

Figure 4.13a

Cell Wall - Structure

- Almost all bacteria can be divided into two large groups based on the levels of peptidoglycan and physical properties of their cell walls.
  - **Gram positive**
  - **Gram negative**



Gram-Positive cell walls

- The cell wall is thick (15-80 nanometers), consisting of **several layers of peptidoglycan**.
- Running perpendicular to the peptidoglycan sheets are a group of molecules called **teichoic acids** which are unique to the Gram-positive cell wall.
  - **Wall teichoic** acid links to peptidoglycan
  - **Lipoteichoic** acid links to plasma membrane

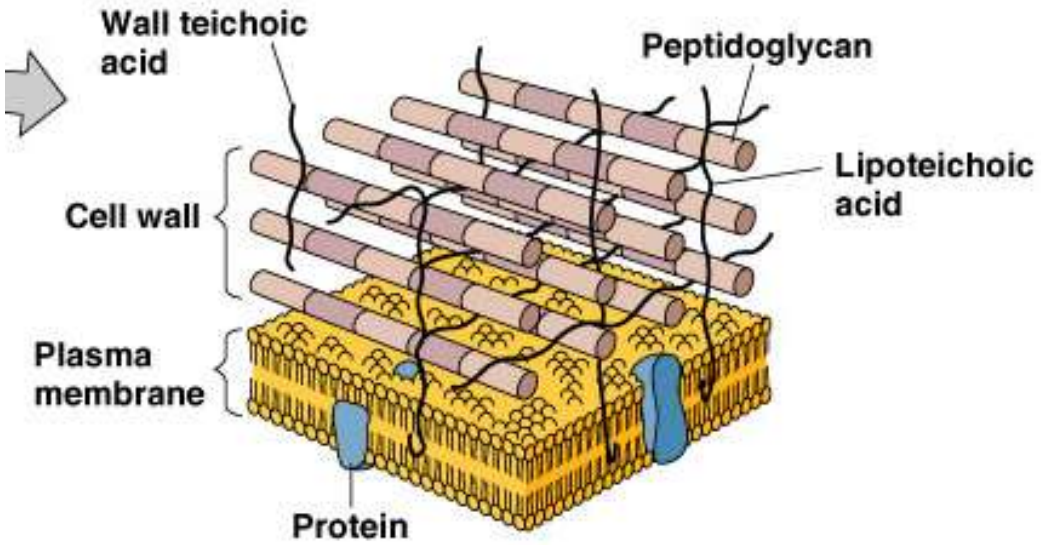
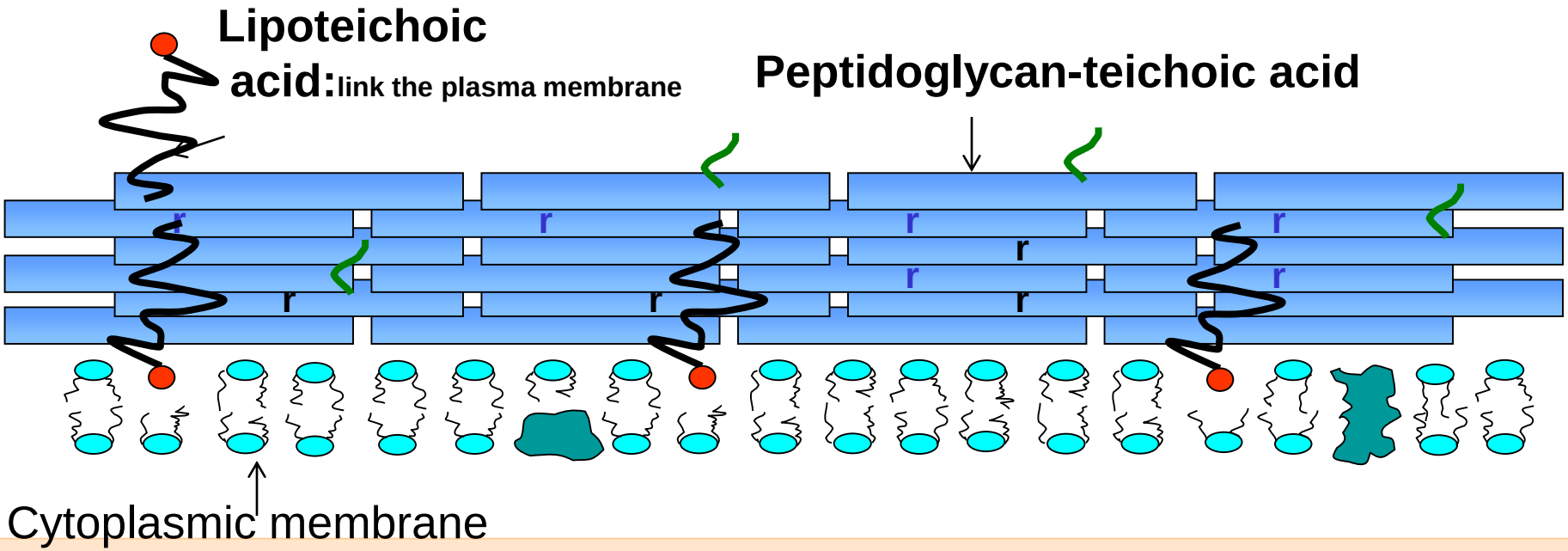


Figure 4.13b

Gram Positive Cell Envelope



Cytoplasm

Gram Positive Cell Envelope

Covalently bound to the thick peptidoglycan are:

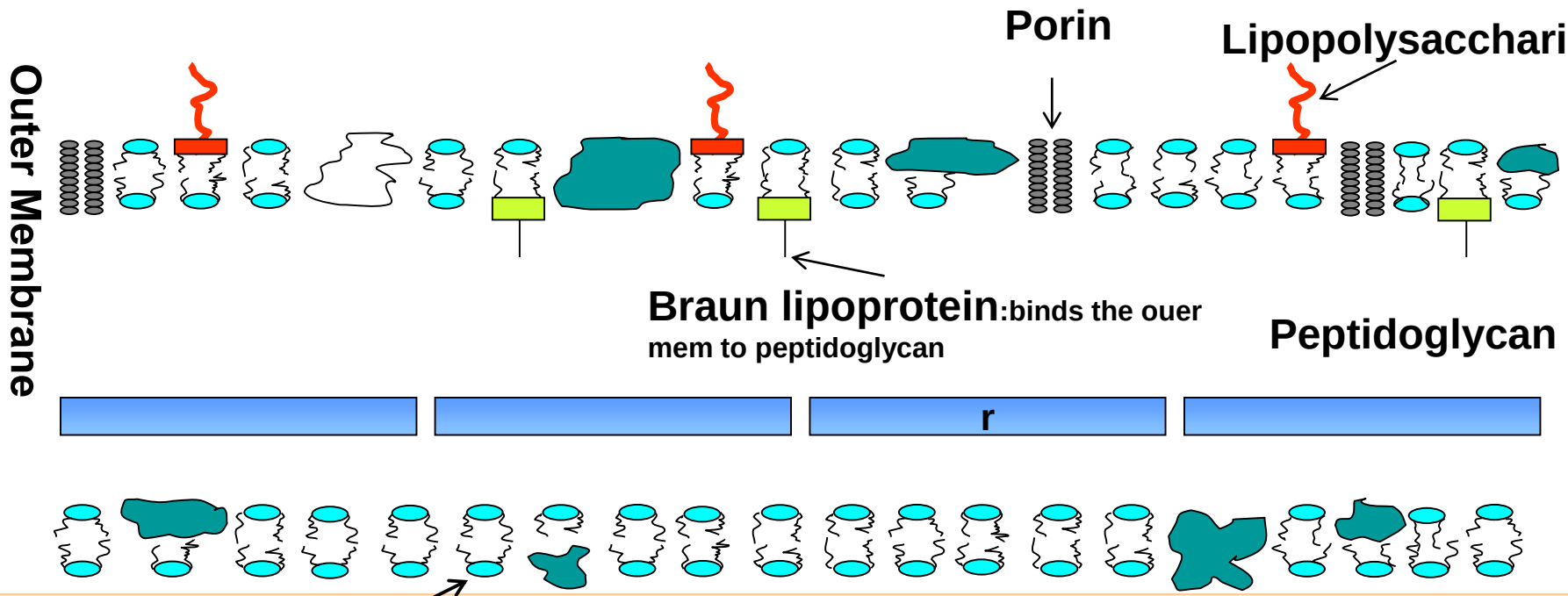
a. Teichoic acid their backbones are usually phosphorus-containing polymers of ribitol or glycerol or

b. Teichuronic acid which are glucuronic acid-containing polysaccharides.

These negatively charged molecules are believed to be involved in concentrating metal ions from the surroundings.

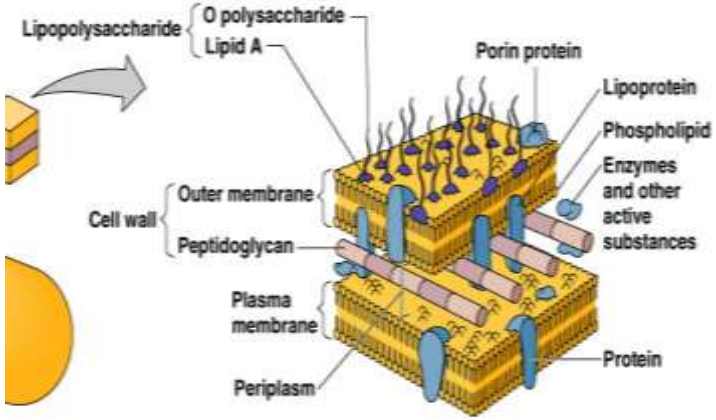
Teichoic acids play crucial roles in cell shape determination, regulation of cell division, and other fundamental aspects of gram-positive bacterial physiology. Additionally, WTAs are important in pathogenesis and play key roles in antibiotic resistance.

Gram Negative Cell Envelope



Gram-Negative cell walls

- The cell wall is relatively thin (10 nanometers) and is composed of:
  1. A **single layer of peptidoglycan**
  2. **Outer membrane - <u>part of the cell wall.</u>**
     - **Outer membrane** composition is distinct from that of the cytoplasmic membrane
     - Unique component, **lipopolysaccharide** (**LPS** or **endotoxin**), which is toxic to animals.
       - **O polysaccharide** part - antigen
       - **Lipid A** - endotoxin
     - **Porins** (proteins) form channels through membrane
     - **Protection** from phagocytes, complement, antibiotics.



3. **Periplasm** – area between the outer membrane and the plasma membrane.

Gram Negative Cell Envelope

Covalently linked to the thin peptidoglycan is

a. **Braun lipoprotein** which binds the outer membrane to the cell wall.

b. **Proteins** and **phospholipids**

c. **Lipopolysaccharide** which consists of three regions:

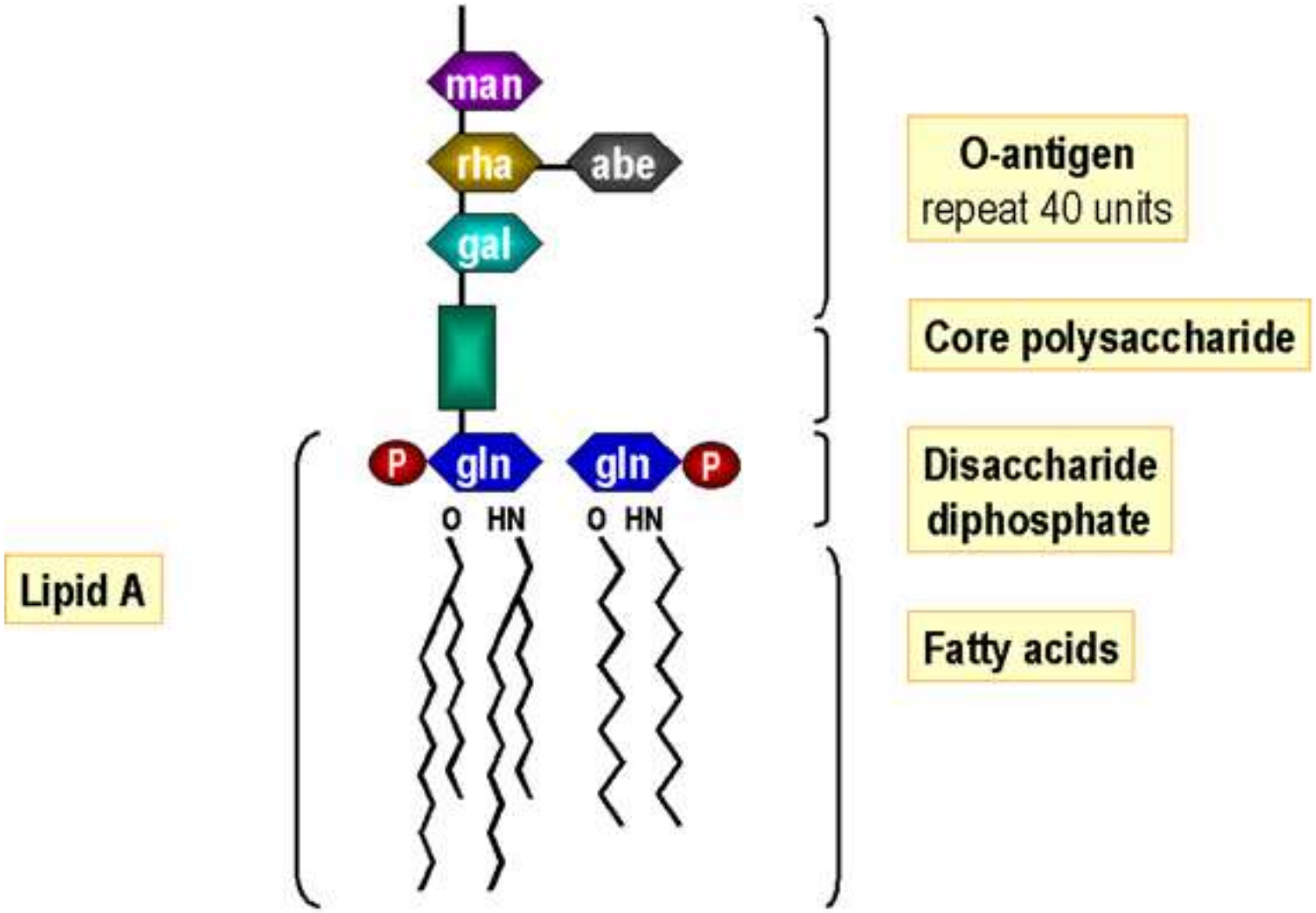
   a. an **outer O antigen**,

   b. a **middle core** which contains several sugars (**heptoses** and **ketodeoxyoctonic acid**), not found elsewhere in nature

   c. an **inner lipid A region** which contains **ß hydroxy fatty acids** (uncommon in nature). The molecule displays endotoxin activity.

Porins in the outer membrane help form channels to allow passage of small hydrophilic nutrients (such as sugars) through the outer membrane.

Structure of Lipopolysaccharide



Atypical Cell Walls

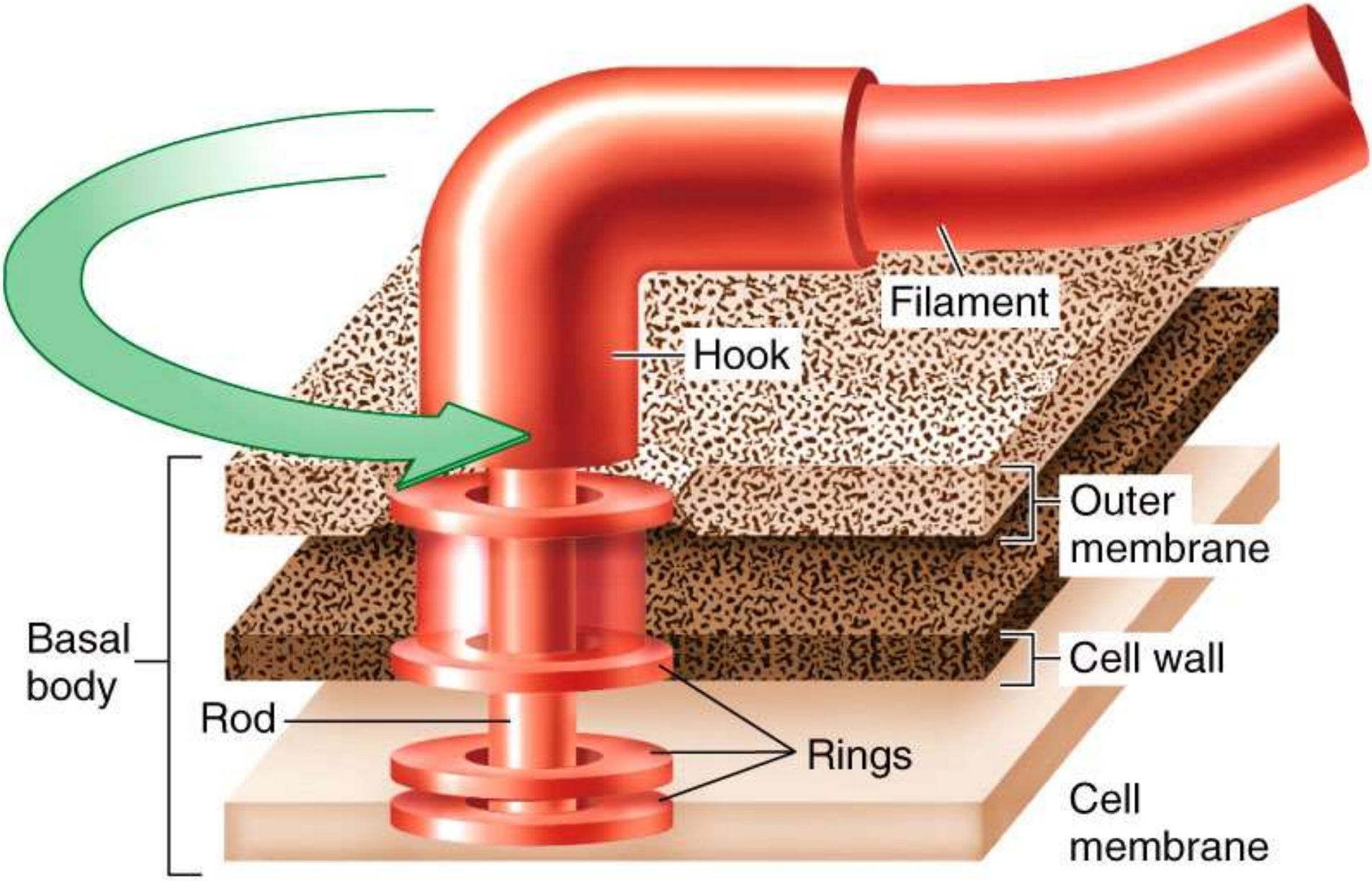
- **Mycoplasmas**
  - **Lack cell walls**
  - Have **sterol-like molecules** incorporated into their membranes and they are usually inhabitants of osmotically-protected environments (contain a high concentration of external solute)
- **Archaea**
  - Walls of pseudomurein (lack NAM and D amino acids)
  - Wall-less

External Structures

- Appendages
  - two major groups of appendages:
    - Motility – flagella and axial filaments (periplasmic flagella)
    - Attachment or channels – fimbriae and pili
- Glycocalyx – surface coating

Flagella

- 3 parts:
  - **filament** – long, thin, helical structure composed of protein **flagellin**
  - **hook**- curved sheath
  - **basal body** – stack of rings firmly anchored in cell wall
- Rotates 360°
- Number and arrangement of flagella is important in classification of bacteria
- Functions in motility of cell through environment



Flagellar Arrangements

1. Monotrichous – single flagellum at one end
2. Lophotrichous – small bunches arising from one end of cell
3. Amphitrichous – flagella at both ends of cell
4. Peritrichous – flagella dispersed over surface of cell

(a)

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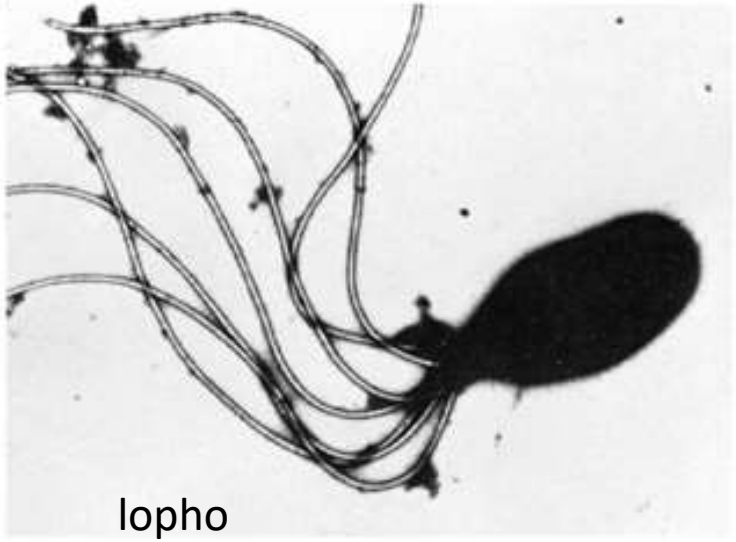

lopho

(b)

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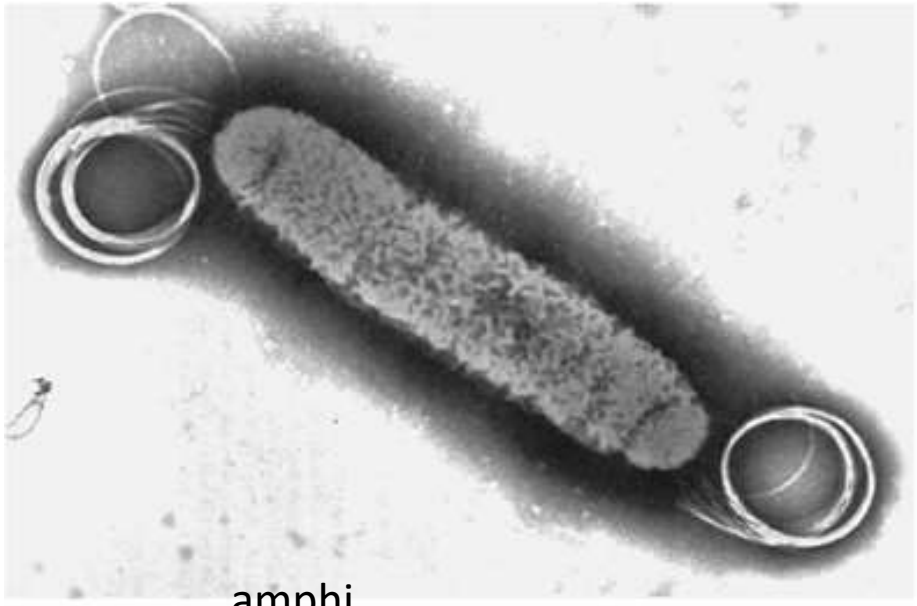

amphi

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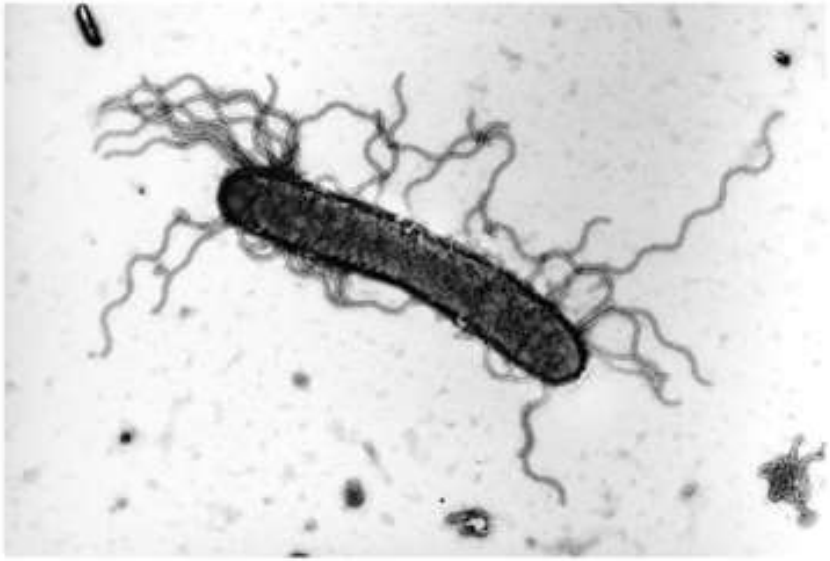

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Flagellar Function

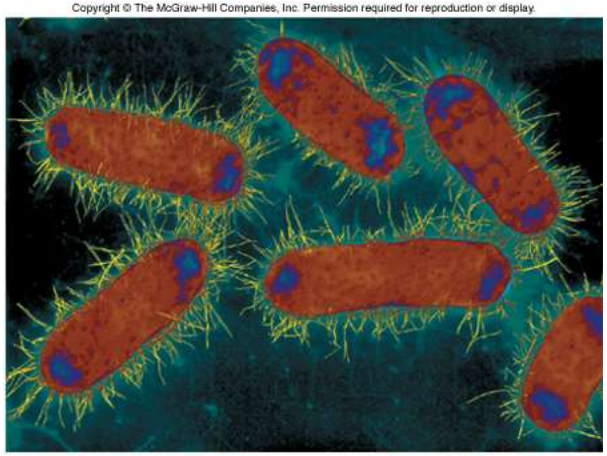
- Guide bacteria in a direction in response to external stimulus:
  1) chemical stimuli – **chemotaxis**; positive and negative
  2) light stimuli – **phototaxis**
- Signal sets flagella into rotary motion clockwise or counterclockwise

Axial Filaments

- Periplasmic, internal flagella, enclosed between cell wall and cell membrane of spirochetes
- Produce cellular motility by contracting and imparting twisting or flexing motion

Fimbriae

- Fine, proteinaceous, hairlike bristles from the cell surface
- Function in adhesion to other cells and surfaces



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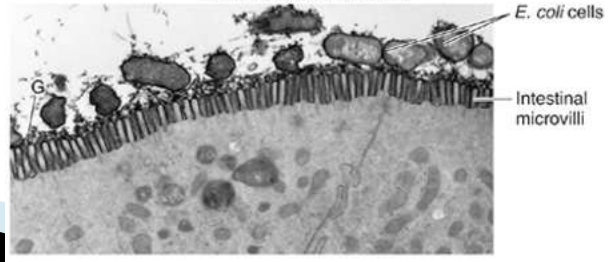
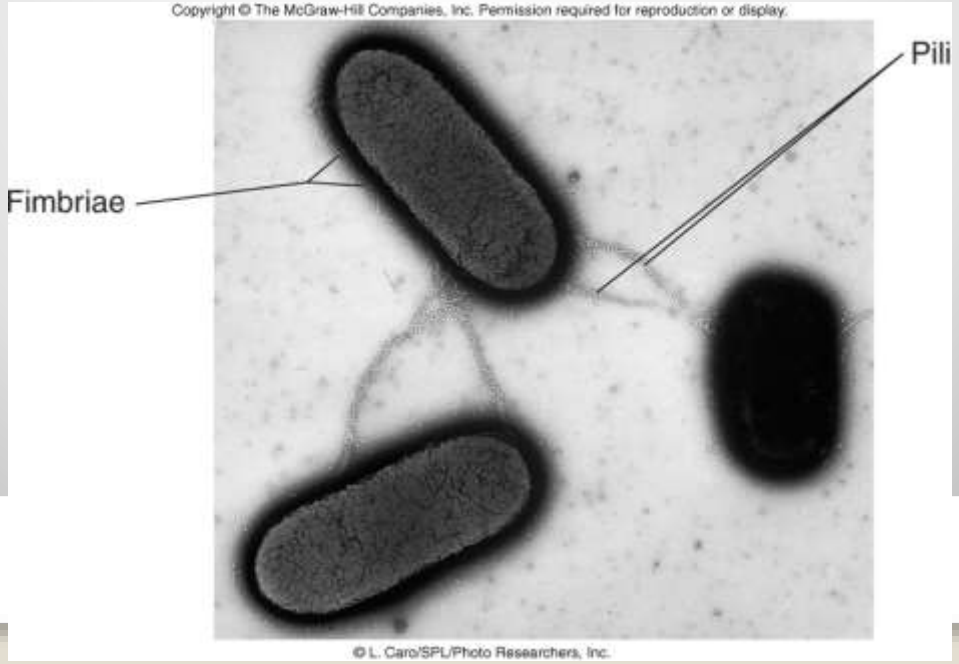


(b)

Pili

- Rigid tubular structure made of **pilin** protein
- Found only in Gram negative cells
- Function to join bacterial cells for partial DNA transfer called **conjugation**



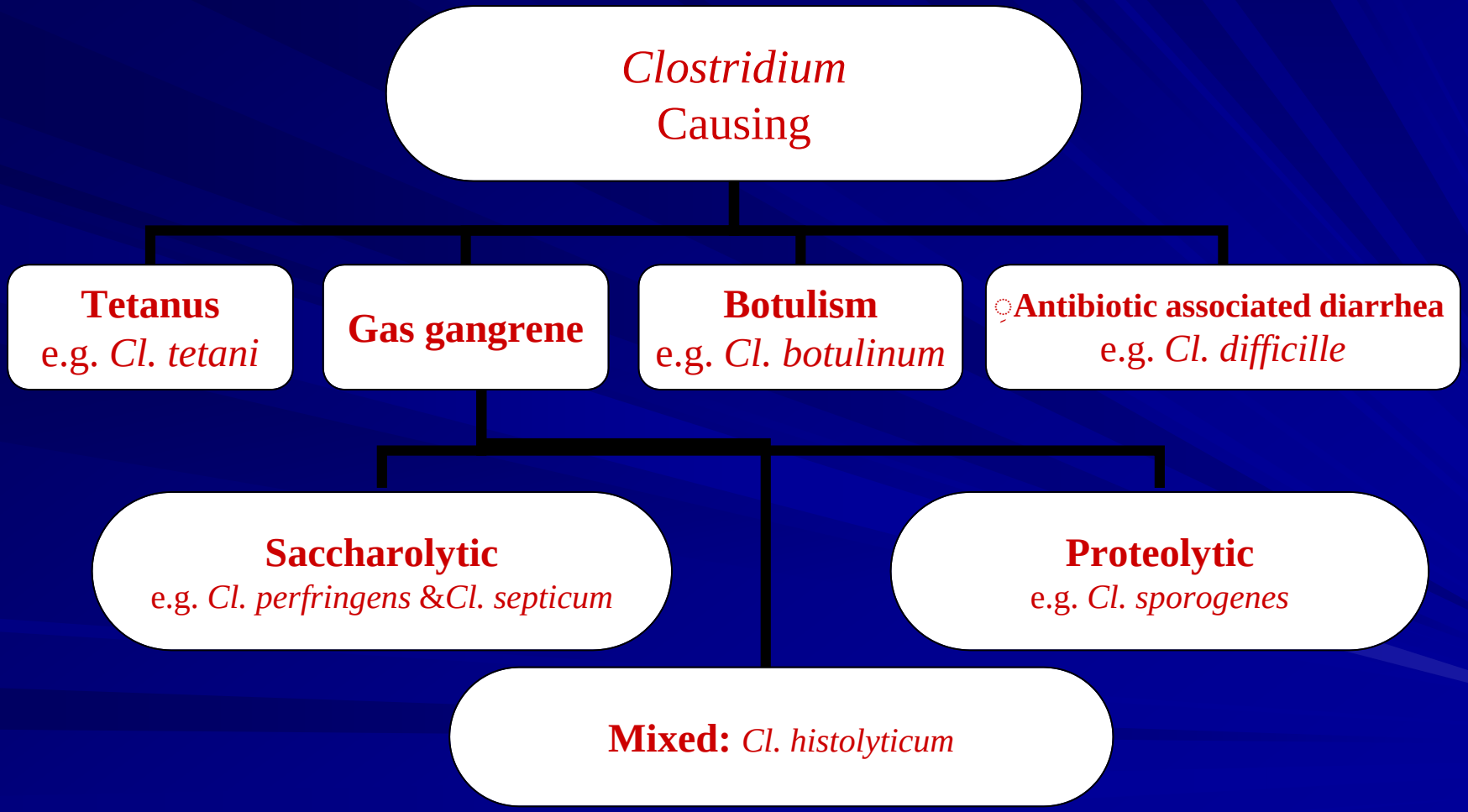
Glycocalyx

- Coating of molecules external to the cell wall, made of sugars and/or proteins
- Two types:
  1. slime layer - loosely organized and attached
  2. capsule - highly organized, tightly attached
- Functions:
  - protect cells from dehydration and nutrient loss
  - inhibit killing by white blood cells by phagocytosis contributing to pathogenicity

Clostridia

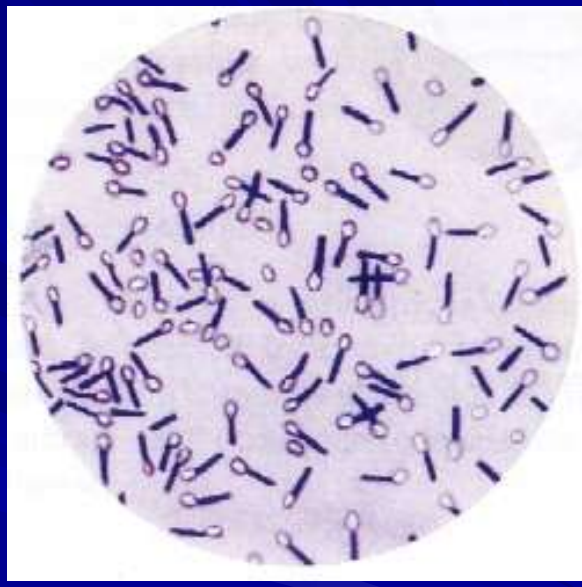
- Large Gram positive
- Straight or slightly curved rods with slightly rounded ends
- Anaerobic bacilli
- Spore bearing
- Spore do not germinate and growth does not normally proceed unless a suitably low redox potential exists
- Saprophytes
- Some are commensals of the animal & human gut which invade the blood and tissue when host die and initiate the decomposition of the corpse (dead body)
- Causes diseases such as gas gangrene, tetanus, botulism & pseudo-membranous colitis by producing toxins which attack the neurons pathways

Clostridia of medical importance



Clostridium Causing Tetanus
Cl. tetani

- Gram positive, straight, slender rod with rounded ends
- All species form endospore (drumstick with a large round end)
- Fermentative
- Obligate anaerobe
- Motile by peritrichous flagella
- Grows well in cooked meat broth and produces a thin spreading film when grown on enriched blood agar
- Spores are highly resistant to adverse conditions
- Iodine (1%) in water is able to kill the spores within a few hours



Toxins

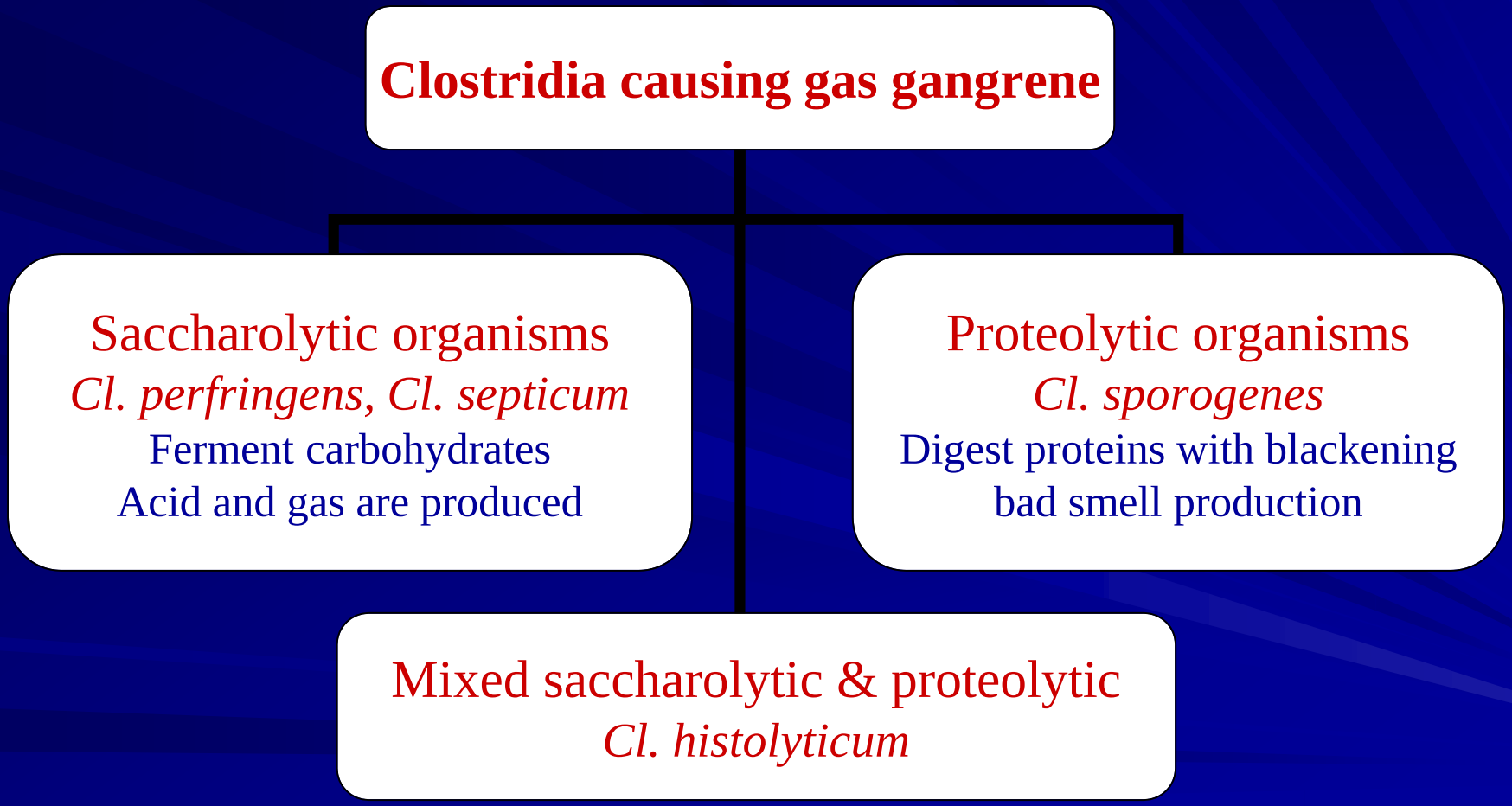
- *Cl. tetani* produces two types of toxins:
  - **Tetanolysin**, which causes lysis of RBCs
  - **Tetanospasmin** is neurotoxin and essential pathogenic product
    - Tetanospasmin is toxic to humans and various animals when injected parenterally, but it is not toxic by the oral route
    - Tetanospasmin which causes increasing excitability of spinal cord neurons and muscle spasm

Laboratory Diagnosis of Tetanus

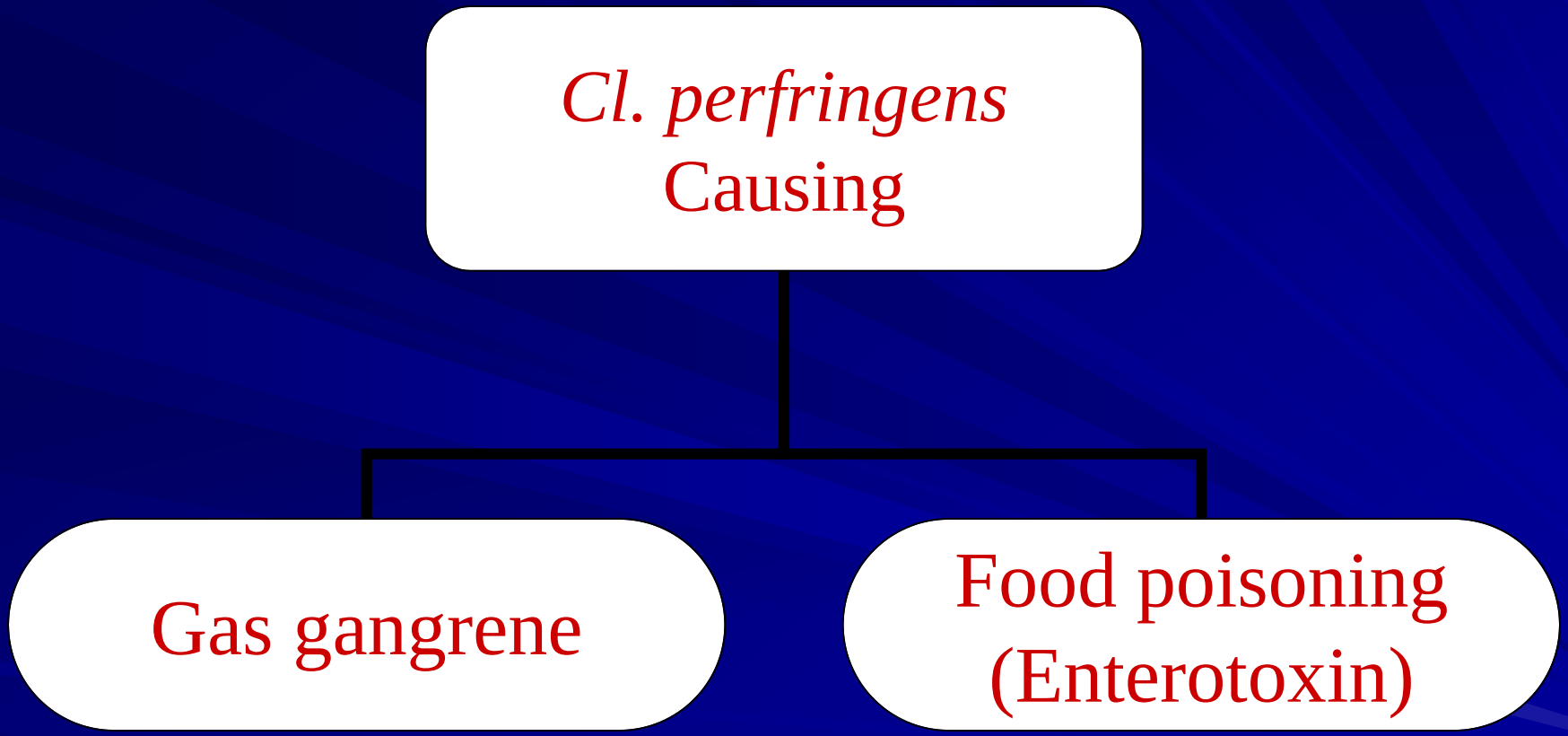
- The diagnosis of tetanus depends primarily upon the clinical manifestation of tetanus including muscle spasm and rigidity.
- **Specimen:** Wound exudates using capillary tube
- **Culture:**
  - On blood agar and incubated anaerobically
  - Growth appears as a fine spreading film.
- Gram stain is a good method for identifying *Clostridium*
  - *Cl. tetani* is Gram positive rod motile with a round terminal spore giving a drumstick appearance

Sir Charles Bell's portrait of a soldier dying of tetanus. The characteristic rigidity of the body is referred to as opisthotonos and risus sardonicus. Original in the Royal College of Surgeons of Edinburgh, Scotland.

Clostridium Causing Gas Gangrene



Saccharolytic Microorganisms



Clostridium perfringens

- Large Gram-positive bacilli with stubby ends
- **Capsulated**
- **Non motile (*Cl. tetani* is motile)**
- Anaerobic
- Grown quickly on selective media
- **Can be identified by** Nagler reaction

Toxins

- **The toxins of *Cl. perfringens***
  - **α toxin** (phospholipase C, lecithinase) is the most important toxin
    - Lyses of RBCs, platelets, leucocytes and endothelial cells
    - Increased vascular permeability with massive hemolysis and bleeding tissue destruction
    - Hepatic toxicity and myocardial dysfunction
  - **β-toxin** is responsible for necrotic lesions in necrotizing enterocolitis
  - **Enterotoxin** is heat labile toxin produced in colon → food poisoning

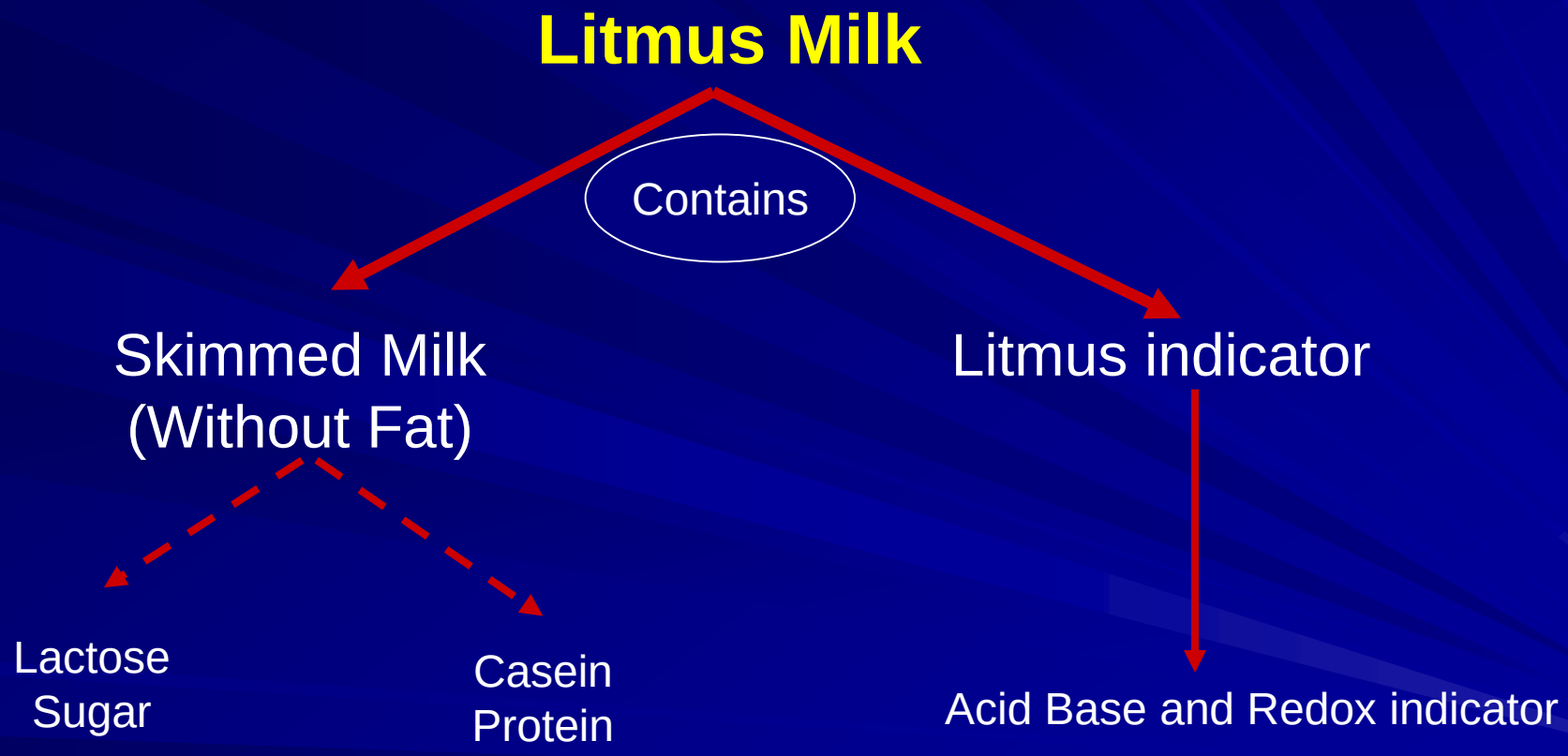
Laboratory Diagnosis

- **Specimen:** Histological specimen or wound exudates
  - Histological specimen transferred aseptically into a sterile screw-capped bottle & used immediately for microscopical examination & culture
  - Specimens of exudates should be taken from the deeper areas of the wound where the infection seems to be most pronounced
- **Microscopical examination (Gram, Spore stain etc)**
  - Gram-positive bacilli, **non motile**, **capsulated** & sporulated
  - The spore is oval, sub-terminal & non bulging
  - Spores are rarely observed
- **Culture:** Anaerobically at 37C
  - **On Robertson's cooked meat medium** → blackening of meat will observed with the production of H_2S and NH_3
  - **On blood agar** → β-hemolytic colonies

Biochemical Tests

- *Cl. perfringnes* characterized by:
  - It ferments many carbohydrates with acid & gas
  - It acidified litmus milk with stormy clot production
  - Nagler reaction is positive

Reaction on Litmus Milk



Reaction on Litmus Milk

1- Acidic Reaction

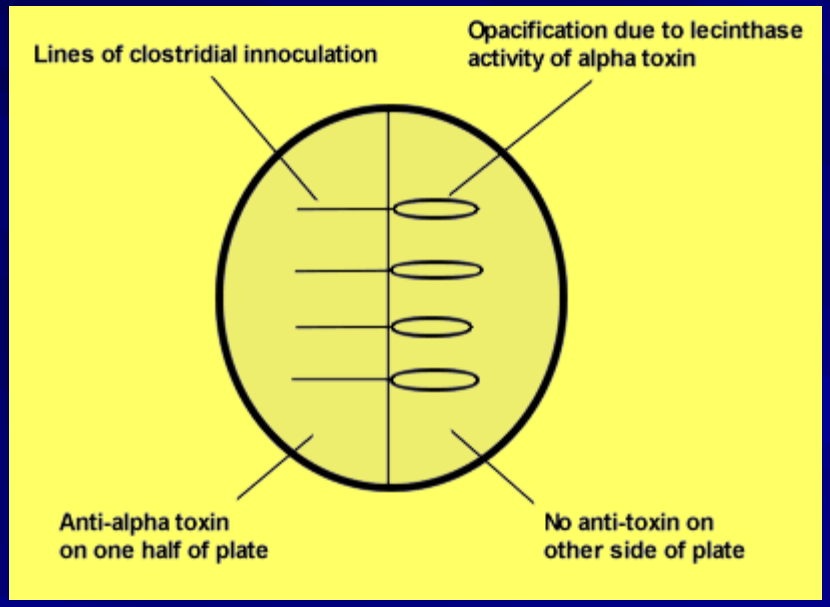
2- Basic Reaction

Reaction on Litmus Milk

Nagler's Reaction

- This test is done to detect the lecithinase activity
  - The M.O is inoculated on the medium containing human serum or egg yolk (contains lecithin)
  - The plate is incubated anaerobically at 37 C for 24 h
  - Colonies of *Cl. perfringens* are surrounded by zones of turbidity due to lecithinase activity and the effect is specifically inhibited if *Cl. perfringens* antiserum containing α antitoxin is present on the medium

Nagler Reaction



Procedure of Nagler Reaction

Positive Nagler Reaction

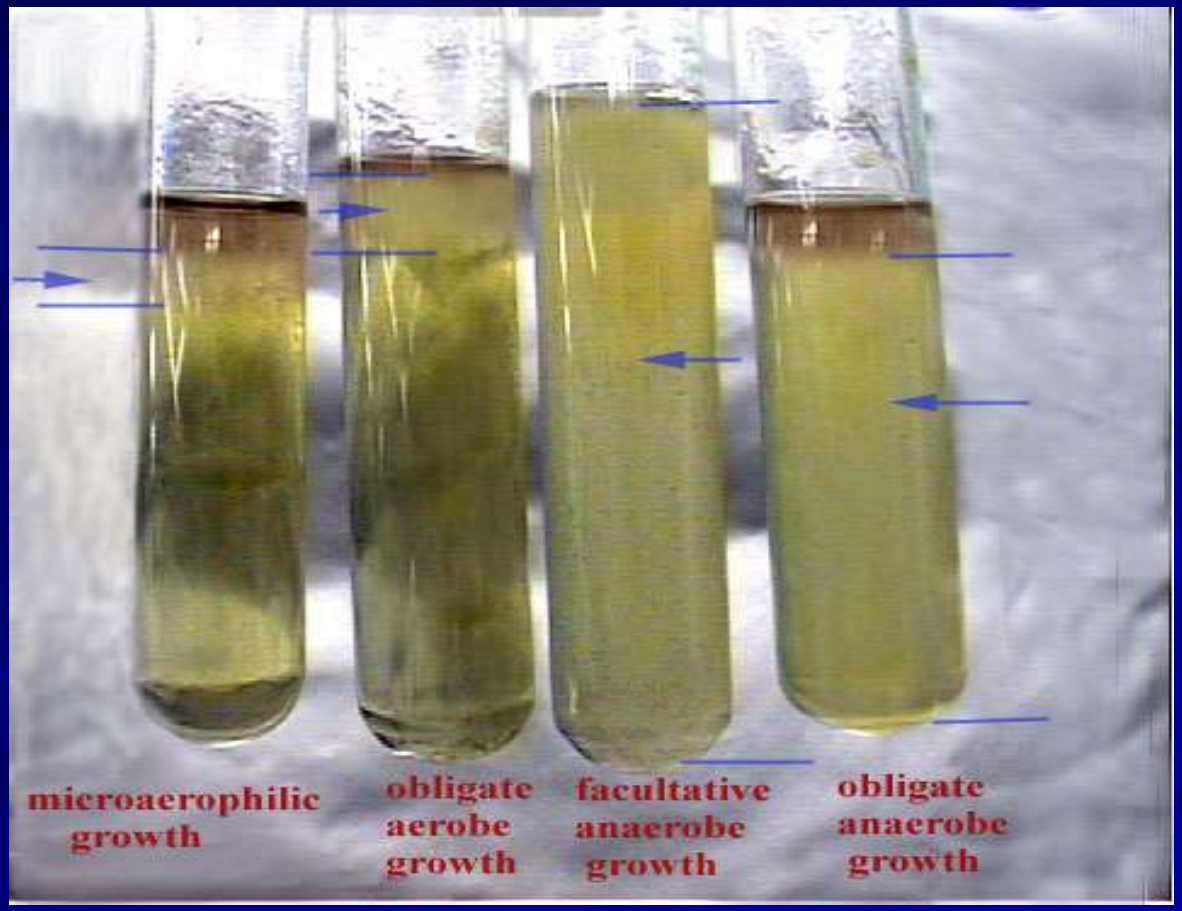
Anaerobic Cultivation

- Removal of oxygen & replacing it with inert gas
  - Anaerobic Jar
    - It is especially plastic jar with a tightly fitted lid
    - Hydrogen is introduced from commercially available hydrogen generators envelop
    - 10 ml of water is added to envelop immediately before placing it in the jar
    - Hydrogen and carbon dioxide will release and react with oxygen in the presence of catalyst to form water droplet
    - Anaerobic indicator (Methylene blue) is placed in the jar
    - Methylene blue is blue in oxidized state (Aerobic condition) while turns colorless in reduced state (Anaerobic condition)

Anaerobic Cultivation

- Culture Media containing reducing agent
  - **Thioglycollate broth**
    - It contains
      - Sodium thioglycollate (Reducing agent)
      - Rezazurin (redox indicator)
      - Low percentage of Agar-Agar to increase viscosity of medium
  - **Cooked Meat Medium**
    - It contains
      - Meat particles (prepared from heart muscles) which contain hematin & glutathione that act as reducing agent

Growth on Fluid Thioglycolate



Clostridium sporogenes Growing in Thioglycolate Medium

Reducing agents in the medium absorb oxygen and allow obligate anaerobes to grow

Reaction on Cooked Meat Medium

- Saccharolytic reaction
  - It causes fermentation of glycogen of muscles
  - Production of acid and gas
  - Meat particles remain intact
  - e.g *Cl. perfergines*
- Proteolytic Reaction
  - It causes digestion of meat particles
  - Formation of black, foul smelling due to sulfur compounds

Anaerobic Jar

Candle Jar

Clostridium botulinum

Physiology and Structure

Gram-positive, spore-forming bacillus.

Strict anaerobe (vegetative cells extremely oxygen-sensitive).

Fastidious growth requirements.

Can produce one of seven distinct botulinum toxins (A–G).

Strains associated with human disease produce lipase, digest milk proteins, hydrolyze gelatin, and ferment glucose.

Virulence

Spore formation.

Botulinum toxin (prevents release of neurotransmitter acetylcholine).

Binary toxin.

Epidemiology

Ubiquitous; *C. botulinum* spores are found in soil worldwide

Human diseases associated with toxins A, B, E, and F.

Relatively few cases of botulism in the United States.

Infant botulism more common than other forms.

Diseases

Foodborne botulism.
Infant botulism.
Wound botulism.

Diagnosis

Botulism confirmed by isolating the organism or detecting the toxin in food products or the patient's feces or serum.

Treatment, Prevention, and Control

Treatment involves administration of metronidazole or penicillin, trivalent botulinum antitoxin, and ventilatory support.

Spore germination in foods prevented by maintaining food in an acid pH, by high sugar content (e.g., fruit preserves), or by storing the foods at 4°C or colder.

Toxin is heat-labile so can be destroyed by heating of food for 20 minutes at 80°C.

Infant botulism is associated with consumption of contaminated foods (particularly honey). Infants younger than 1 year should not be given honey or foods containing it.

C. botulinum — **agent of botulism,** a rare, but severe (lethal) neuroparalytic disease

Morphology and Physiology

- heterogeneous group of fastidious, strictly anaerobic bacilli
- **motile by peritrichous flagella**
- heat-resistant **spores (ovoid, subterminal)**
- proteolytic and non-proteolytic

Antigenic Structure

- species divided into **four groups (I-IV)** based on type of toxin produced and proteolytic activity
- **seven antigenically distinct botulinum toxins (types A to G)**
- somatic antigens - heat stable and heat labile; spore antigens - more specific

Pathogenicity Determinants

- **lethal foodbome intoxication with toxin types A,B,E,or F**; shorter incubation period, poor prognosis
- phage-mediated, systemic-acting A-B neurotoxin (botulinum toxin = botulin) released at cell lysis
  - **Mode of Action** - one of most extremely potent neurotoxins known
    (1 ng of purified toxin contains about 200,000 minimal lethal doses (MLDs) for a 20g mouse)
    - **A-B toxin ingested, binds specific receptors on peripheral cholinergic nerve endings (neuromuscular junctions) where it blocks release of presynaptic acetylcholine (excitatory neurotransmitter) blocking muscle stimulation & resulting in flaccid paralysis**
    - **Early**: nausea, vomiting, weakness, lassitude (lack of energy), dizziness, constipation
    - **Later**: double vision, difficulty in swallowing and speaking
    - Final: death due to respiratory paralysis

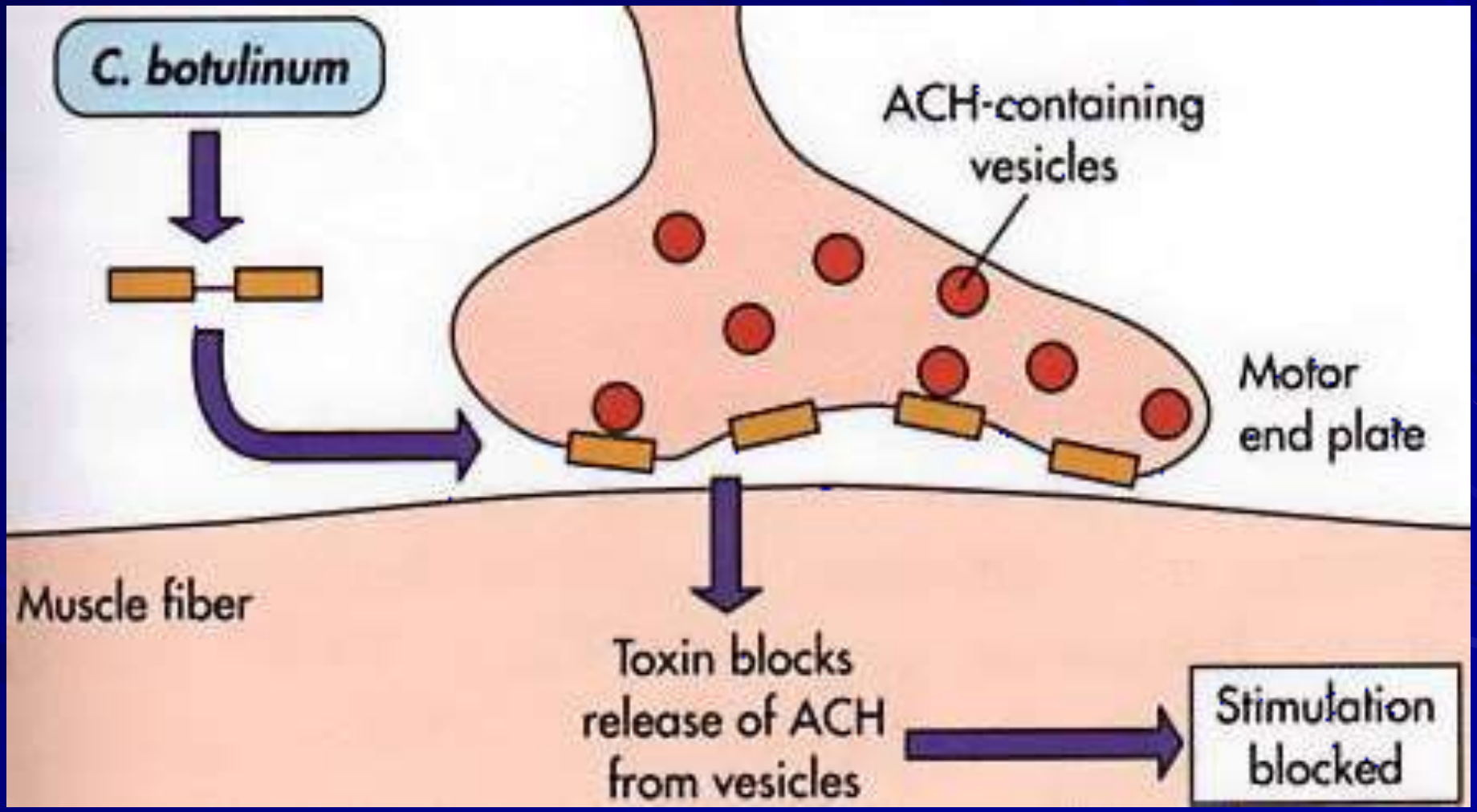
Lab Identification

- microscopic detection or Cx (culture) are often unsuccessful (few organisms and slow growing)
- toxin detected and typed in lab via toxicity and antitoxin neutralization tests in mice or by ELISA

Diagnosis/Treatment/Prevention

- **crucial to rapidly diagnose** (symptoms often confusing); note the type of botulinum toxin involved
- Tx (treatment) should be administered as quickly as possible on basis of clinical Dx (diagnosis)
  - **ventilatory support & trivalent (A, B, E) antitoxin (polyvalent) binds free toxin in bloodstream**
  - administer **gastric lavage & metronidazole or penicillin eliminates organisms from GI tract**
  - **care in home canning** and in heating of home-canned food; toxoid is available

Mechanism of Action of Botulinum Toxin



Diphtheroids

have traditionally been considered part of the normal commensal flora of the skin and mucous membranes of the respiratory tract, urinary tract and conjuctiva.

In fact, about 12-30% of humans carry C. urealyticum as part of their normal skin flora.

Named for their resemblance to corynebacterium diphtheria

- Gram positive bacillus bacteria with varied shape and low virulence
- Non-toxin producers, (–OID means non-pathogenic) so they have no toxin.
- Responsible for body odor caused by the bacterial breakdown of sweat.
- Common diphtheroid is propionibacterium acnes.

Diphtheroids are cultured on Mueller-Hinton-Tellurite (MHT) plates. If diphtheroids are present, they will produce gray or black colonies because they are able to reduce the tellurite in the media to tellurium, which appears as a gray or black precipitate.

Propionibacterium acnes

- is the relatively slow-growing, typically aerotolerant anaerobic, Gram-positive bacterium (rod) linked to the skin condition of acne;
- This bacterium is largely commensal and part of the skin flora present on most healthy adult humans' skin.
- It is usually just barely detectable on the skin of healthy preadolescents.
- It lives primarily on, among other things, fatty acids in sebum secreted by sebaceous glands in the follicles.
- It may also be found throughout the gastrointestinal tract in humans and many other animals.

P. acnes bacteria live deep within follicles and pores, away from the surface of the skin.

In these follicles, *P. acnes* bacteria use sebum, cellular debris and metabolic byproducts from the surrounding skin tissue as their primary sources of energy and nutrients.

Elevated production of sebum by hyperactive sebaceous glands (sebaceous hyperplasia) or blockage of the follicle can cause *P. acnes* bacteria to grow and multiply.

- *P. acnes* bacteria secrete many proteins, including several digestive enzymes.
- These enzymes are involved in the digestion of sebum and the acquisition of other nutrients.
- They can also destabilize the layers of cells that form the walls of the follicle.
- The cellular damage, metabolic byproducts and bacterial debris produced by the rapid growth of *P. acnes* in follicles can trigger inflammation.
- This inflammation can lead to the symptoms associated with some common skin disorders, such as **folliculitis** and **acne vulgaris**

➢The damage caused by *P. acnes* and the associated inflammation make the affected tissue more susceptible to colonization by opportunistic bacteria, such as ***Staphylococcus aureus***

➢*P. acnes* has also been found in corneal ulcers, and is a common cause of chronic endophthalmitis

➢Rarely, it infects heart valves leading to endocarditis

➢*P. acnes* has been found in herniated discs. The propionic acid which it secretes creates micro-fractures of the surrounding bone. These micro-fractures are sensitive and it has been found that antibiotics have been helpful in resolving this type of low back pain

❖*P. acnes* bacteria are susceptible to a wide range of antimicrobial molecules, from both pharmaceutical and natural sources.

❖Antibiotics are commonly used to treat infections caused by *P. acnes*.

❖Acne vulgaris is the disease most commonly associated with *P. acnes* infection.

❖The antibiotics most frequently used to treat acne vulgaris are: **erythromycin, clindamycin, doxycycline** and **minocycline**

❖The antibiotic families that *P. acnes* are most likely to acquire resistance to are the **macrolides** (e.g., erythromycin and azithromycin), **lincosamides** (e.g., clindamycin) and **tetracyclines** (e.g., doxycycline and minocycline)

Listeria

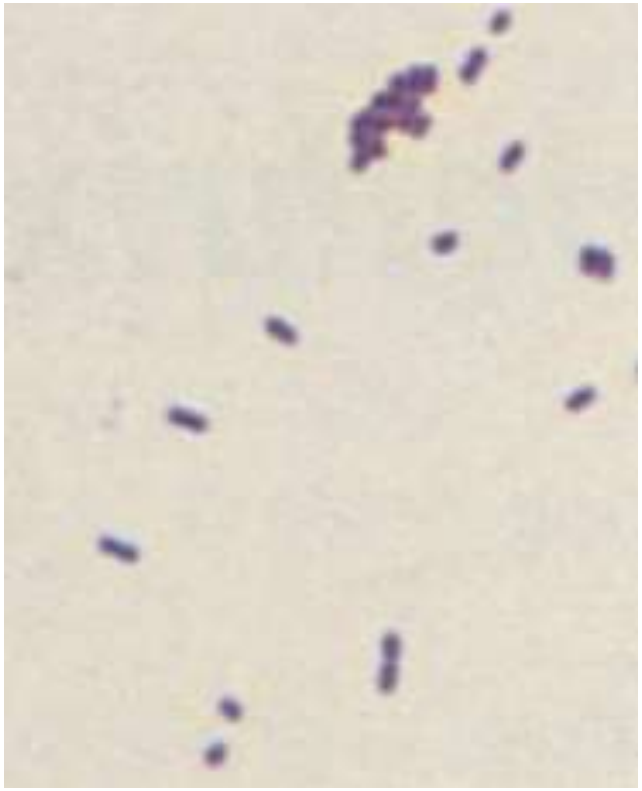
L. monocytogenes: meningitis and bacteremia

Structure and Physiology

Small gram-positive coccobacilli, facultative anaerobic.

Motile at room temperature but not at 37 ^{o}C.

Grow on most conventional media in a wide pH range.cold temperatures.



L. monocytogenes

Pathogenesis and Immunity

Widely distributed in nature (soil, water, vegetation, and the intestines of a variety of animals). Fecal carriage in healthy people: 1%-5%.

Human disease is restricted to neonates and the elderly, pregnant women, and immunocompromised patients (particularly those with defective cell-mediated immunity, such as AIDS patients).

L. monocytogenes

Clinical Diseases

Neonates

Early onset disease (acquired transplacentally in utero): granulomatosis infantiseptica, with disseminated abscesses and granulomas in multiple organs.

Late onset disease (acquired at or soon after birth): meningitis or meningoencephalitis with septicemia, similar to that caused by group B streptococci.

Adults

Healthy

- Asymptomatic or mild influenza-like illness.
- Gastrointestinal symptoms in some patients.

Immunocompromised

- Meningitis (high risk: organ transplant patients, cancer patients, pregnant women)
- Primary bacteremia: chills and fever; high fever and hypotension in severe cases. Maybe fatal.

L. monocytogenes

Laboratory Diagnosis

Specimen: CSF and blood.

Gram stain: CSF typically show no *Listeria* because of the low bacterial concentration.

Culture

Listeria grows on most conventional media.

Traditionally food and environmental samples are enriched in a broth prior to subculture into a further broth and then onto selective agar.

- The initial broth incubation is at 30˚C for 24 hours, the subsequent broth incubation is at 35˚C for 24 hours. All broth cultures are then subcultured onto agar for a further 24 hours and subsequently identified by biochemical tests (it take about 5 days).

Selective media and enrichment are used for specimens contaminated with rapidly growing bacteria.

- Hemolysis (β-) and motility in liquid or semisolid medium are useful for preliminary identification.

Identification

Biochemical and serological tests.

1. identified using the Microbact™ Listeria 12L System.
2. Serological tests

- ❑ Serological tests for the detection of antibodies have not been traditionally used for the diagnosis of listeriosis.
- ❑ They have been largely unreliable, because:
- it lacking sensitivity and specificity.
- ❑ A number of formats, including ELISA, complement fixation and microagglutination have been largely unsuccessful in the diagnosis of culture-proven human listeriosis, because:
- even in the absence of immunosuppression Considerable cross-reactivity with antigenic determinants of other Gram-positive organisms has been observed.

- On the other hand, L. monocytogenes is a ubiquitous organism, and regular exposure of animals and humans to this microorganism is very common.
- Many healthy individuals are intestinal carriers (2–6%) and anti-L. monocytogenes serum antibody prevalence as high as 53% have been reported in humans.
- immunoassay; molecular methods such as PCR

L. monocytogenes

Treatment, Prevention, and Control

L. monocytogenes is resistant to multiple antibiotics (e.g., cephalosporin and tetracycline). Currently, penicillin or ampicillin, either alone or with gentamicin, is the treatment of choice.

Outbreaks have been associated with the consumption of contaminated milk, soft cheese, undercooked meat, unwashed raw vegetables, and cabbage. Refrigeration of contaminated food products permits the slow multiplication of the organisms to an infectious dose.

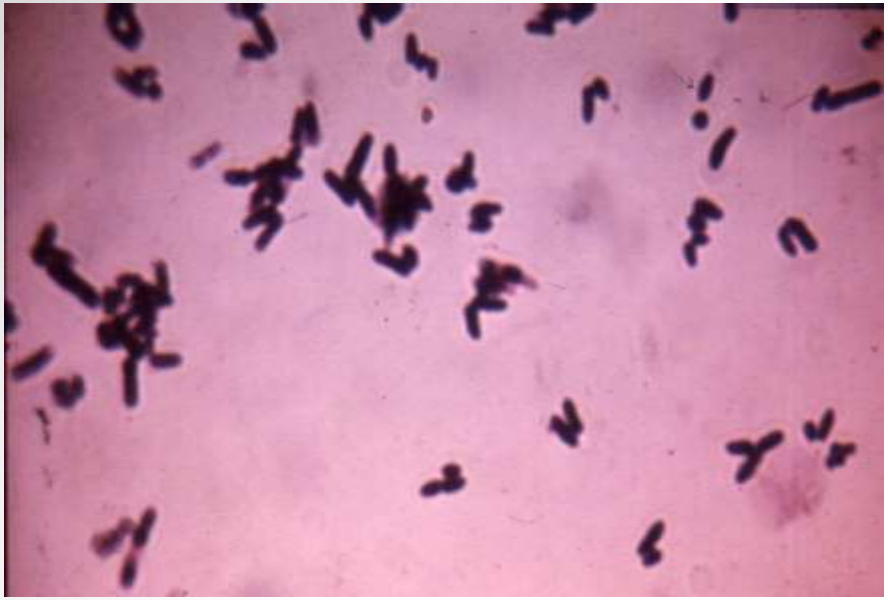
Because *Listeria* organisms are ubiquitous and most infections are sporadic, prevention and control are difficult. High risk people should avoid eating raw or partially cooked foods.

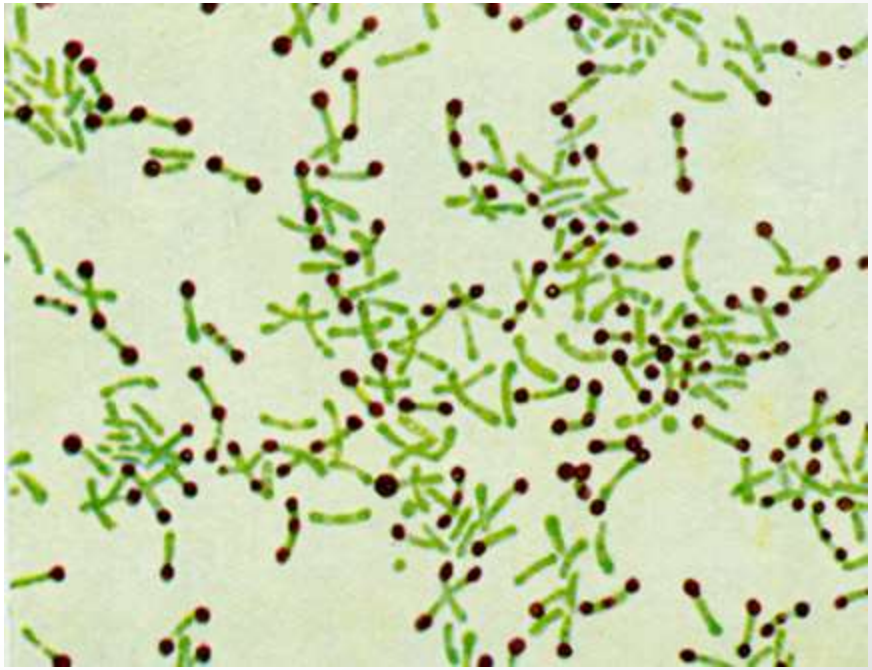
Diphtheria

- Diphtheria (Greek διφθέρα (diphthera) "pair of leather scrolls") is an upper respiratory tract illness caused by *Corynebacterium diphtheriae*, a facultative anaerobic Gram-positive bacterium.

Morphoy

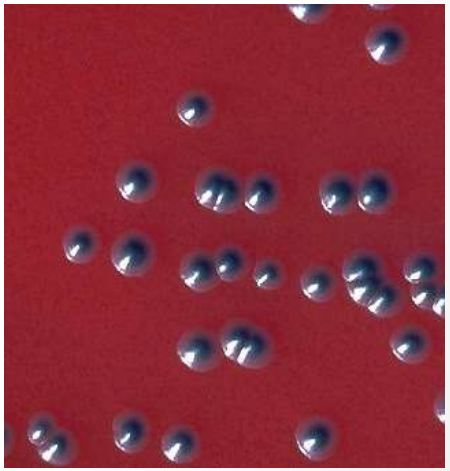
- Gram positive bacilli. 3-6 μ x 0.5-0.8 μ.
- v or k or L shape.
- Chinese letter pattern, angular arrangement
- Metachromatic granules. volutin granules, polymetaphosphate energy storage depots
- Alberts stain – green and bluish black
- Nonmotile noncapsulated, nonsporing
- pleomorphic

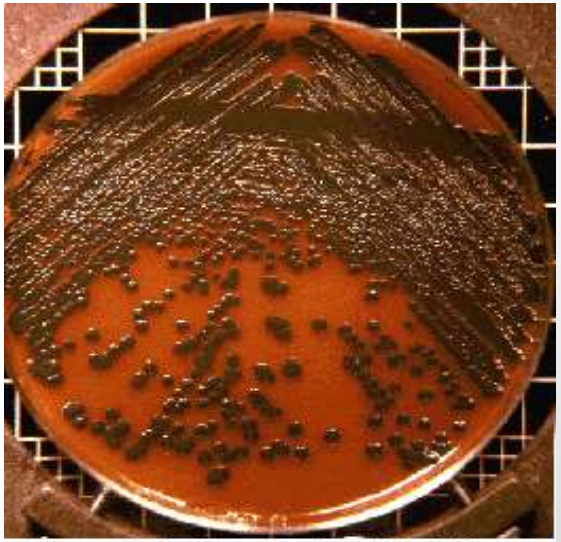




Cultivation

- Loefflers serum slope– creamy white colonies in 6-8 hrs
- Potassium tellurite medium—black colonies
- Blood agar: revealed small, greyish, smooth, non-hemolytic colonies.



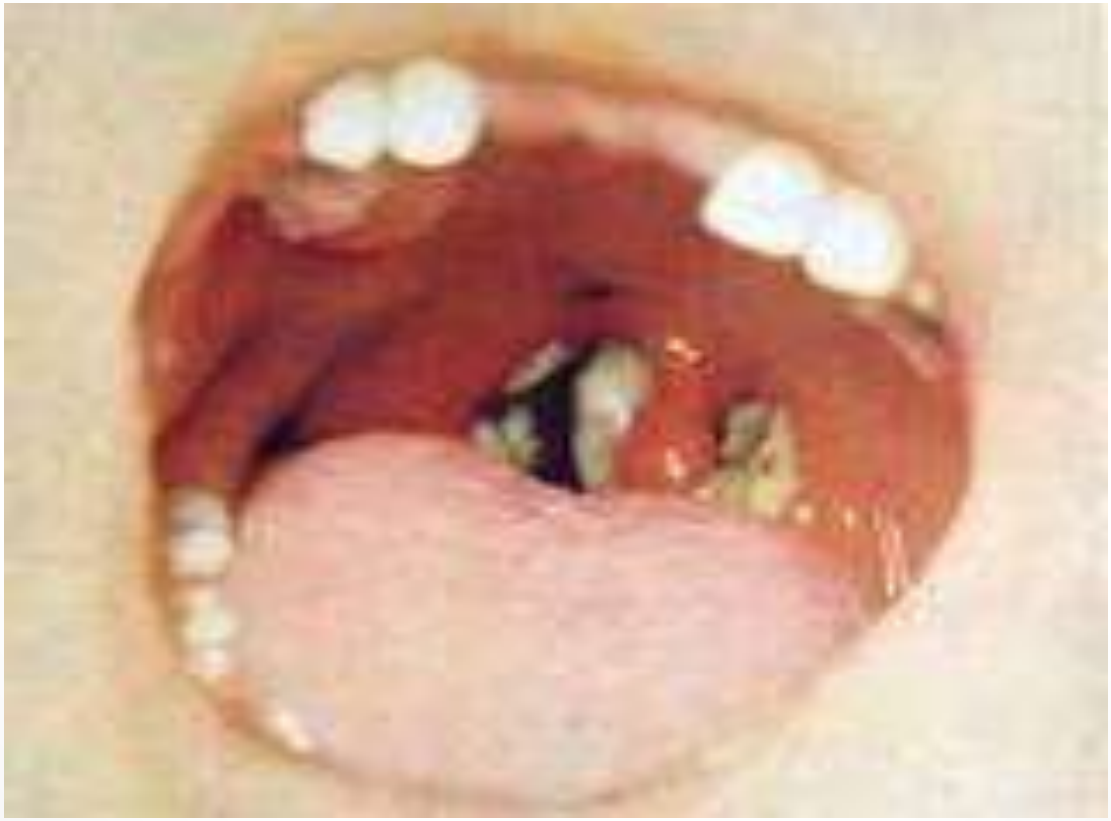


Corynebacterium pseudodiphtheriticum
Chocolate tellurite agar

Biochemical reactions

- Ferments glucose ,maltose with acid only
- Lactose, sucrose, mannitol not fermented
- Urease negative

- It is characterized by sore throat, low fever, and an adherent membrane (a pseudomembrane) on the tonsils, pharynx, and/or nasal cavity.



Mode of Transmission:

- Diphtheria pathogenesis most often occurs due to respiratory or skin transmission, which later develops into tissue necrosis, which is mediated by its toxin.
- Diphtheria is a contagious disease spread by direct physical contact or breathing the aerosolized secretions of infected individuals.
- Humans are the only known reservoir of *C. diphtheriae*.
- Person-to-person transmission occurs through oral or respiratory droplets, close physical contact, and rarely, by fomites.
- discharge from skin lesions may transmit infection in cutaneous diphtheria.

Epidemiology

- The disease first noticed in 1878 when queen victoria's daughter get infected.
- In the 1920s there were an estimated 100,000 to 200,000 cases of diphtheria per year in the United States, causing 13,000 to 15,000 deaths per year with a case fatality ratio of about 7.5%.
- In 1884 Friedrich Loeffler discovered the causative organism (*Corynebacterium diphtheriae*).
- As the vaccine developed by Emil von Behring in 1913 deaths began declining in earnest in 1924.
- During the 1990s, large epidemics occurred in the newly independent states of the former Soviet Union.
- In temperate area respiratory diphtheria is common, while in many but not all tropical area cutaneous diphtheria is more common.

Biotypes

1)gravis(13 types)-most serious disease

Colonies: large, irregular, gray

2)mitis(40 types)-mild illness

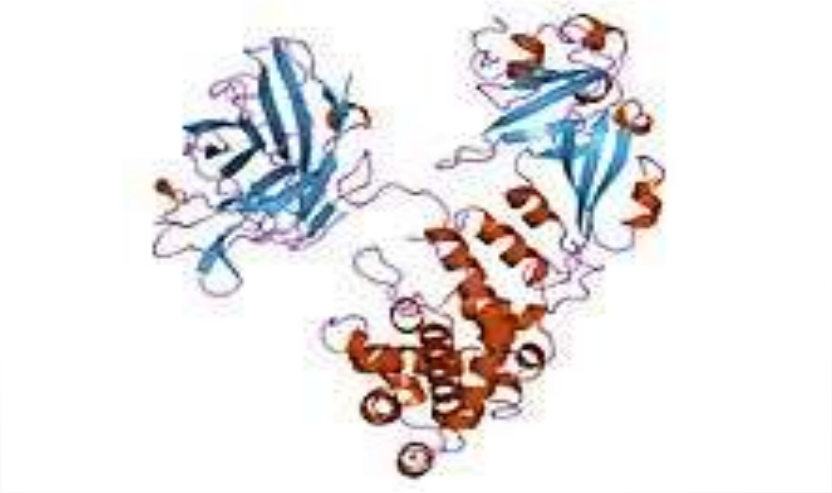
colonies: small, round, convex, black

3)intermedius(4 types)-intermediate severity

Colonies: small, flat and gray

Diphtheria toxin

- Is an exotoxin gaining entry into the cell cytoplasm and inhibiting protein synthesis.
- It is a single polypeptide chain of 535 amino acids consisting of two subunits linked by disulfide bridges. Binding to the cell surface of the less stable of these two subunits allows the more stable part of the protein to penetrate the host cell.



Mechanism

- Diphtheria toxin is a single protein of 60000 MW. composed of two fragments: fragment B that allows entry into host cells and fragment A that prevents the host cell from making proteins. The toxin binds to a cell-surface receptor to gain entry into the cell.
- Diphtheria toxin is produced by *C. diphtheriae* only when it is infected with a bacteriophage that integrates the toxin-encoding genetic elements into the bacteria.

Diphtheria

- Children, fatal if not treated in time
- Exclusively human disease
- Droplet infection- fomites
- Fever, cervical lymphadenopathy, pseudomembrane
- Myocarditis arrhythmia ⟶ fatal
- Polyneuropathy, palatine paralysis
- Rare in adults.

Clinical classification

i) Malignant (hypertoxic) diphtheria

Signs: severe toxemia and adenitis, lymph glands swelling in the neck

Complications: death-circulatory failure, paralytic sequelae

ii) Septic diphtheria:

Signs: ulceration with pseudomembrane formation and cellulites

iii) Hemorrhagic diphtheria

Signs: local and general bleeding from edge of psudomembrane, conjunctival, epistaxis and purpura

Complications

1)Asphyxia-obstruction of resp tract

2)Acute circulatory failure

3)kidney failure

4)paralysis-soft palate, eye muscles, extremities (3rd-4th week)

5)septic sequelae-pnemonia, otitis media

DIAGNOSIS

Laboratory criteria

- Isolation of *Corynebacterium diphtheriae* from a clinical specimen.

Clinical criteria

- Upper respiratory tract illness with sore throat
- Low-grade fever (>103°F is rare)
- An adherent pseudomembrane of the tonsil(s), pharynx, and/or nose.

Laboratory diagnosis

- Sample collection: Throat swab or swab from membrane
- Microscopy: Gram stain and Alberts stain
- Culture: Loefflers and PT
- Biochemicals
- Virulence test in vivo and in vitro

Treatment:

- Intubation and tracheotomy to manage the obstruction in the throat.
- Diphtheria antitoxin should be given on based on clinical diagnosis and should not wait for laboratory confirmation.
- Metronidazole
- Erythromycin (orally or by injection) for 14 days (40 mg/kg per day with a maximum of 2 g/d), or
- Procaine penicillin G given intramuscularly for 14 days (300,000 U/d for patients weighing <10 kg and 600,000 U/d for those weighing >10 kg). Patients with allergies to penicillin G or erythromycin can use rifampin or clindamycin

Prevention

a) DTP (DPT)-

- Formal toxoid - Incubation of Toxin at pH 7.4 -7.6 for 3 – 4 weeks at 37 °C
- Adsorbed toxoid – purified toxoid adsorbed on to aluminum phosphate or hydroxide
- triple vaccine given to children.

  trivalent preparation
- **Diphtheria toxoid, Tetanus toxoid and Pertussis vaccine**

Td- contains absorbed ***tetanus*** and ten-fold smaller dose of ***diphtheria toxoid*.**

b) Schedule

i) primary immunization –

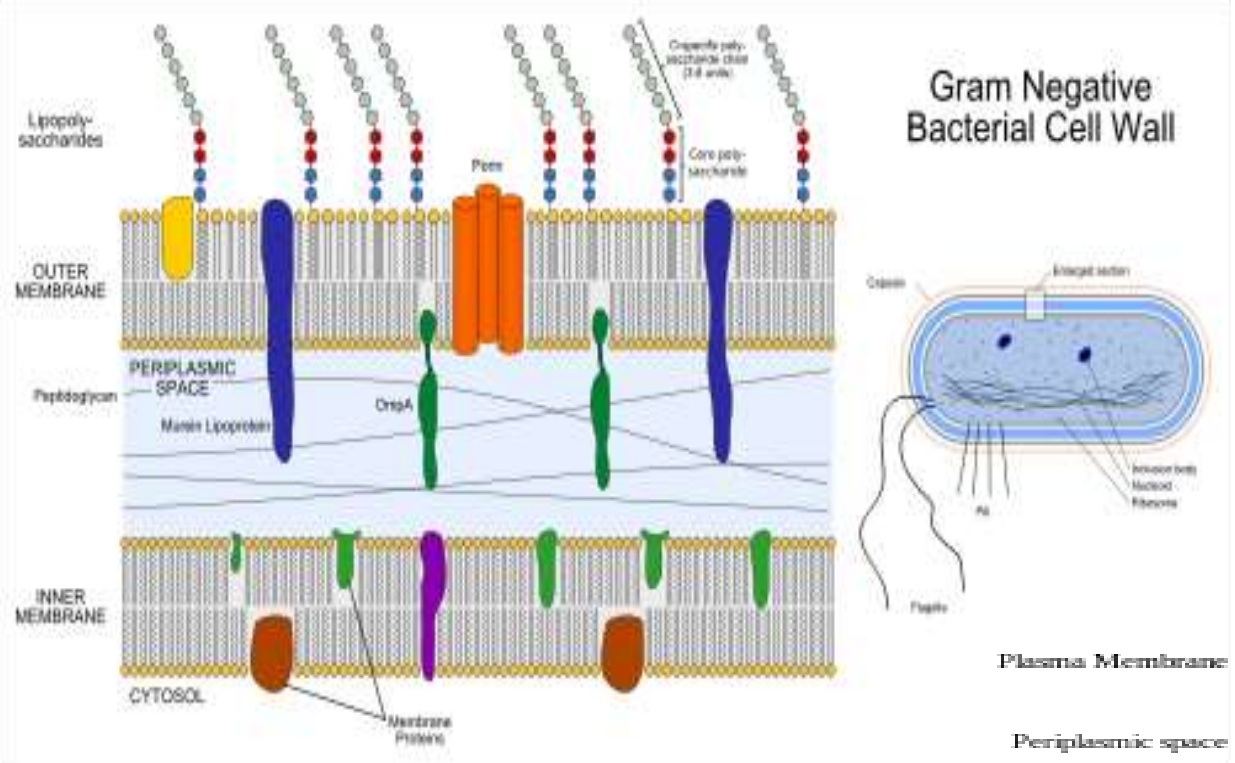
- infants and children
  - 3 doses, 4-6 weeks
  - 4th dose after a year
  - booster at school entry

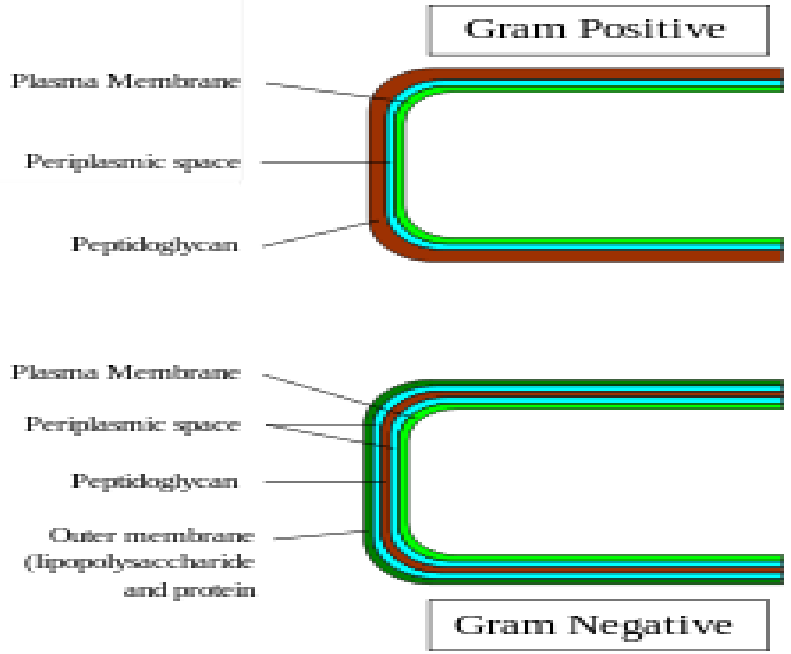
ii) Booster immunization

- adults
- Td toxoids used (traveling adults may need more)

Gram-negative bacteria

- are a class of bacteria that do not retain the crystal violet stain used in the Gram staining method of bacterial differentiation.
- The thin peptidoglycan layer of their cell wall is sandwiched between an inner cell membrane and a bacterial outer membrane.
- In Gram staining, the outer lipid-based membrane of gram-negative bacteria is removed by an alcohol solution.
- The alcohol also decolorizes the thin exposed peptidoglycan layer by dissolving away the previously applied crystal violet.
- A counterstain (safranin or fuchsine) is then added which recolorizes the bacteria red or pink.


Gram Negative
Bacterial Cell Wall
Lipopoly-
saccharides
Core poly-
saccharide
Porin
OUTER
MEMBRANE
PERIPLASMIC
SPACE
Peptidoglycan
OmpA
Murein Lipoprotein
INNER
MEMBRANE
CYTOSOL
Membrane
Proteins


Gram Positive
Plasma Membrane
Periplasmic space
Peptidoglycan
Plasma Membrane
Periplasmic space
Peptidoglycan
Outer membrane
(lipopolysaccharide
and protein
Gram Negative

Gram-negative bacteria display the following characteristics:

1. Cell membrane (cytoplasmic).

2. Thin peptidoglycan layer (which is much thicker in gram-positive bacteria)

3. Outer membrane containing lipopolysaccharide (LPS, which consists of lipid A, core polysaccharide, and O antigen) in its outer leaflet and phospholipids in the inner leaflet

4. Porins exist in the outer membrane, which act like pores for particular molecules

5. There is a space between the peptidoglycan layer and the secondary cell membrane called the periplasmic space

6. The S-layer is directly attached to the outer membrane rather than the peptidoglycan

1. If present, flagella have four supporting rings instead of two
2. No teichoic acids or lipoteichoic acids are present
3. Lipoproteins are attached to the polysaccharide backbone.
4. Some of them contain Braun's lipoprotein, which serves as a link between the outer membrane and the peptidoglycan chain by a covalent bond
5. Most, with very few exceptions, do not form spores.
6. Release some endotoxin

- The proteobacteria are a major group of gram-negative bacteria, including *Escherichia coli* (*E. coli*), *Salmonella*, *Shigella*, and other Enterobacteriaceae, *Pseudomonas*, *Moraxella*, *Helicobacter*, *Bdellovibrio*, acetic acid bacteria, *Legionella* etc.

- Other notable groups of gram-negative bacteria include the cyanobacteria, spirochaetes, green sulfur, and green non-sulfur bacteria.

- Medically relevant gram-negative cocci include the three organisms that cause a sexually transmitted disease (*Neisseria gonorrhoeae*), a meningitis (*Neisseria meningitidis*), and respiratory symptoms (*Moraxella catarrhalis*).

➢Medically relevant gram-negative bacilli include a multitude of species. Some of them cause primarily respiratory problems (*Hemophilus influenzae*, *Klebsiella pneumoniae*, *Legionella pneumophila*, *Pseudomonas aeruginosa*), primarily urinary problems (*Escherichia coli*, *Proteus mirabilis*, *Enterobacter cloacae*, *Serratia marcescens*), and primarily gastrointestinal problems (*Helicobacter pylori*, *Salmonella enteritidis*, *Salmonella typhi*).

➢Gram-negative bacteria associated with hospital-acquired infections include *Acinetobacter baumannii*, which cause bacteremia, secondary meningitis, and ventilator-associated pneumonia in hospital intensive-care units.

Gram-negative rods (Enterobacteriaceae):

- Natural habitat is the intestinal tract of humans and animals.
- Some enteric organisms (sometimes called coliform), eg, Escherichia coli, are part of the normal flora and incidentally cause disease.
- They are facultative Anaerobes or aerobes.
- Ferment a wide range of carbohydrates.
- Possess a complex antigenic structure.
- Produce a variety of toxins and other virulence factors.
- Morphologically, highly variable, Capsules are large and regular in klebsiella, less so in enterobacter, and uncommon in the other species.
- In culture, they form circular, convex, smooth colonies with distinct edges (E. coli), mucoid (Enterobacter and Klebsiella).

Classification

Rapid, presumptive identification of gram-negative enteric bacteria depends on their ability to ferment lactose.

1.Lactose Fermented Rapidly: e.g. Escherichia coli, Enterobacter aerogenes, Klebsiella pneumonia.

2. Lactose Fermented Slowly: e.g. Edwardsiella, Serratia, citrobacter, Arizona, Providencia, Erwinia.

3. Lactose Not Fermented: Shigella species, Salmonella species, Proteus species, Pseudomonas species.

Recently Enterobacteriaceae classified into tribes, genera and species according to their cultural and biochemical characters. The species are further classified into biotypes, bacteriophage type and colicin types. Five tribes can be identified now:

1.Tribe I: Escherichia (genus: Eschrichia, Edwardsiella, citrobacter, salmonella and shigella)

2.Tribe II: Klebsielleae (genus: Klebsiella, Enterobacter, Hafina and Serratia)

3. Tribe III: Proteae (genus: Proteus)

4. Tribe VI: Erwinieae (genus: Erwinia)
5. Tribe V: Yersineae (genus: Yersinia pestis, Yersinia enterolitico and Yersinia pseudotuberculosis).

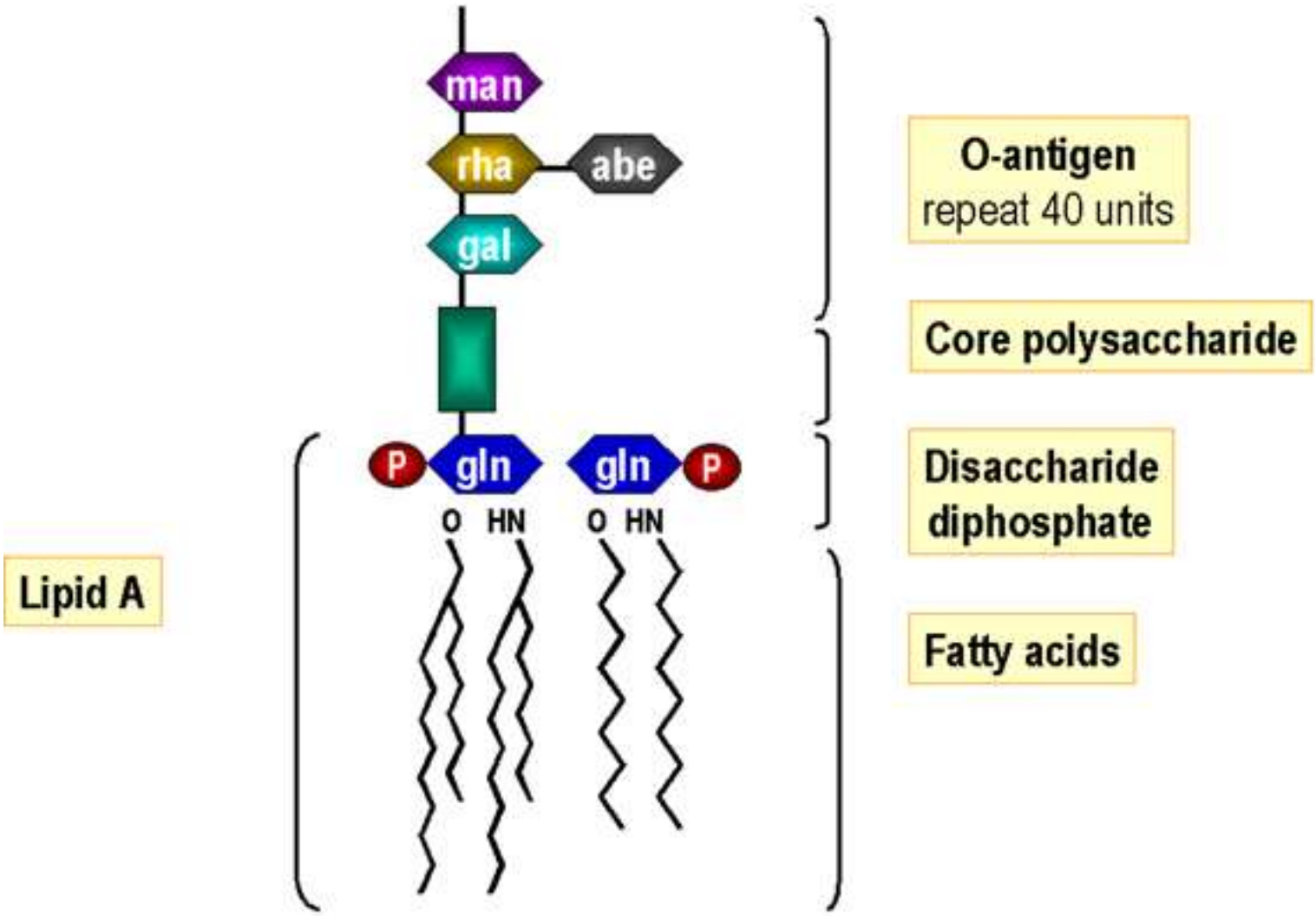
ANTIGENIC STRUCTURE:

Enterobacteriaceae has a complex antigenic structure. The following are the major types of antigens:

a.Somatic O (lipopolysaccharide) antigens: there are about 150 different heat stable O antigen the most external part of the cell wall lipopolysaccharide. O antigens are resistant to heat and alcohol and usually are detected by bacterial agglutination. Antibodies to O antigens are predominantly IgM.

b. K antigens: there are more than 100 heat-labile K antigen which is external to O antigens on some but not all Enterobacteriaceae. It is either polysaccharides or proteins. K antigens may interfere with agglutination by O antisera, and they may be associated with virulence.

Structure of Lipopolysaccharide



c. H antigens: there are more than 50 H antigen (located on flagella and are denatured or removed by heat or alcohol. Such H antigens agglutinate with anti-H antibodies, mainly IgG. Within a single serotype, flagellar antigens may be present in either or both of two forms,

1. Phase 1 (conventionally designated by lower-case letters).

2. Phase 2 (conventionally designated by Arabic numerals).

The antigenic classification of Enterobacteriaceae often indicates the presence of each specific antigen. Thus, the antigenic formula of an E coli may be O55:K5:H21; that of Salmonella Schottmülleri is O1,4,5,12:Hb:1,2.

ESCHERICHIA COLI

Commonly abbreviated E. coli is a Gram-negative, rod-shaped bacterium that is commonly found in the lower intestine of human or animals. Most E. coli strains are harmless, but some serotypes can cause serious food poisoning in humans, and are occasionally responsible for product recalls due to food contamination. The harmless strains are part of the normal flora of the gut, and can benefit their hosts by producing vitamin K2, and by preventing the establishment of pathogenic bacteria within the intestine. and fecal–oral transmission is the major route through which pathogenic strains of the bacteria cause disease.

Morphology

E. coli is Gram-negative, facultative anaerobic and non-sporulating. Cells are typically rod-shaped, and are about 2.0 microns(μm) long and 0.5 μm in diameter, with a cell volume of 0.6–0.7 (μm). Generation time is about 20 minutes.

1.liquid broth culture: after incubation for 24 hours, The culture should be turbid (cloudy) and contain 10^8 and 10^9 cells/mL.

2. nuterient agar: The results displayed small colonies that were circular in shape, a diameter of approximately 0.5mm, the elevation of the colonies was seen to be slightly raised to the surface incredibly smooth.

3. MacConkey agar: the colonies were seen to have a pink colour pigmentation in MacConkey agar due to its lactose fermenter after growth.

4. Blood agar: An isolate from urine can be quickly identified as E coli by its β-hemolysis.

Lactose fermentation

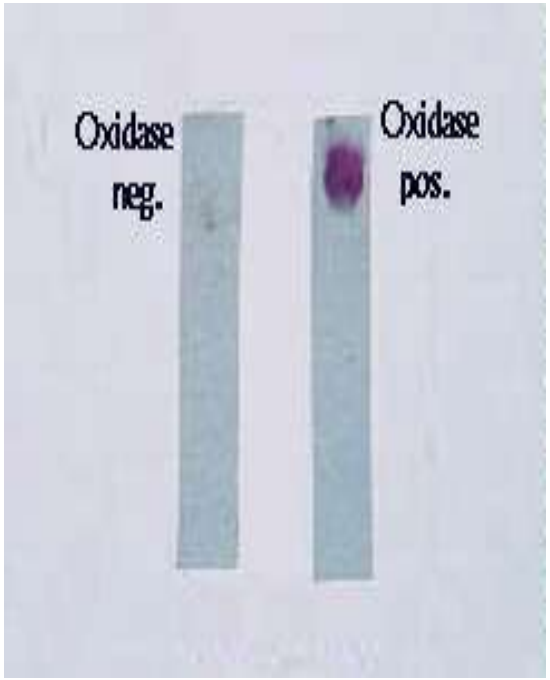
MacConkey agar plate shows the classic picture of Escherichia coli bacterium that turns the agar a lavender color.

Beta-hemolysis

On this plate, the organisms are producing red blood cell hemolysins that completely destroy the nearby red cells and render the agar clear and transparent near the colonies.

Biochemical reactions

E coli typically produces positive tests for indole, lysine decarboxylase, and mannitol fermentation and produces gas from glucose. Typical colonial morphology with an iridescent “sheen” on differential media such as EMB agar, and a positive spot indole test, negative oxidase tests.



Indole test
Right: positive test, *proteus*

Antigenic structure

a. Somatic O (lipopolysaccharide) antigens: there 164 groups designd as 1,2,3, and so on

b. Surface K antegine: they are of 3 types (L antigen, A capsular antigen, B antigen) usually only one is presented. K antigen appears to be important in the pathogenesis of upper tract infection.

c. H or flagellar antigen

d. Fimbrial antigen: no significance in antigenic classification.

Diseases caused by E. coli

It is a member of the normal intestinal flora generally do not cause disease, and in the intestine they may even contribute to normal function and nutrition. The bacteria become pathogenic only when they reach tissues outside of their normal intestinal or other less common normal flora sites. The most frequent sites of clinically important infection are the urinary tract, biliary tract, and other sites in the abdominal cavity, but any anatomic site (eg, bacteremia, prostate gland, lung, bone, meninges) can be the site of disease.

Pathogenesis & Clinical Findings

The clinical manifestations of infections with E coli and the other enteric bacteria depend on the site of the infection and cannot be differentiated by symptoms or signs from processes caused by other bacteria.

1. Urinary tract infection—account for 90% of first urinary tract infections in young women. The symptoms and signs include urinary frequency, dysuria, hematuria, and pyuria. Flank pain is associated with upper tract infection.
2. E coli-associated diarrheal diseases— These E coli are classified by the characteristics of their virulence properties:

a. Enteropathogenic E coli (EPEC): cause diarrhea in infants, as they adhere to the mucosal cells of the small bowel result in watery diarrhea, which is usually self limited but can be chronic. It is associated with multiple serotypes identified by O antigen and occasionally by H antigen typing.

b. Enterotoxigenic E coli (ETEC): cause "traveler's diarrhea" it produce two type of enterotoxins:

1. Heat-labile exotoxin (LT)(MW 80,000) that is under the genetic control of a plasmid and consist of two subunits B attaches to the epithelial cells of the small intestine and facilitates the entry of subunit A (MW 26,000) into the cell. results in hypersecretion of water and chlorides and inhibits the reabsorption of sodium.

2. Heat-stable enterotoxin STa (MW 1500–4000), which is under the genetic control of a heterogeneous group of plasmids. STa activates guanylyl cyclase in enteric epithelial cells and stimulates fluid secretion. Many STa-positive strains also produce LT. The strains with both toxins produce a more severe diarrhea.

c. Enterohemorrhagic E coli (EHEC): associated with hemorrhagic colitis, a severe form of diarrhea, and and with hemolytic uremic syndrome, a disease resulting in acute renal failure, microangiopathic hemolytic anemia, and thrombocytopenia. EHEC produces verotoxin, named for its cytotoxic effect on Vero cells with at least two antigenic forms of the toxin. The most common types produce the virotoxin is O157:H7.

d. Enteroinvasive E coli (EIEC): produces a disease very similar to shigellosis. The disease occurs most commonly in children in developing countries and in travelers to these countries. They cause disease by invading intestinal mucosal epithelial cells.

e. Enteroaggregative E coli (EAEC) causes acute and chronic diarrhea (> 14 days in duration) in persons in developing countries. These organisms also are the cause of food-borne illnesses in industrialized countries. They are characterized by their characteristic pattern of adherence to human cells. EAEC produce ST-like toxin (see above) and a hemolysin.

3. Sepsis—When normal host defenses are inadequate, E coli may reach the bloodstream and cause sepsis. Newborns may be highly susceptible to E coli sepsis because they lack IgM antibodies. Sepsis may occur secondary to urinary tract infection.

4. Meningitis—E coli and group B streptococci are the leading causes of meningitis in infants.

Diagnosis

1. **Hematological investigation:**

a. **Total WBC count usually within normal limits, with slightly or moderate leukocytosis in association to tissue invasion.**

b. **Differntial count reavel increase in polymorphoneuclear cells associated with tissue invasion.**

2. Bacteriological investigation:

Specimen: Urine, blood, pus, spinal fluid, sputum, or other material as indicated by the localization of the disease process.

Smear examination: centrifuged urine deposit is examined for pus cell, RBCs and bacteria. Gram stain shows G-ve bacillus. The stained fecal material is of little use, while using fluorescent-labelled O-group antisera is useful for diagnosis. ELISA, precipitin test, RIA are another useful test to establish the identification of infection.

Culture:

Specimens are plated on both blood agar and differential MacConkey media. With differential media, rapid preliminary identification of gram-negative, lactose fermenter, motile, indole positive and citrate [illegible] bacteria is often possible.

Treatment:

It is sensitive to sulfonamides, trimethoprim, tetracyclines, chloramphenicol and aminoglygosides. Resistance to one or more [illegible]

Vaccine:

is prepared against colonization factor antigen (CFA) which include CFA II, IV and I and it is under evaleuation in Sweden.

Growth curve

By Dr. Zainab Al-Hakeem
Immunologist in microbiology

The growth of microorganisms

Bacterial "growth" means an increase in the number of individuals, not an increase in cell size.

Growth is the orderly increase in all the components of an organism such as size and/or population number.

The rule for bacteria growth can be described as single cell dynamics and population dynamics

Growth: single cell dynamics

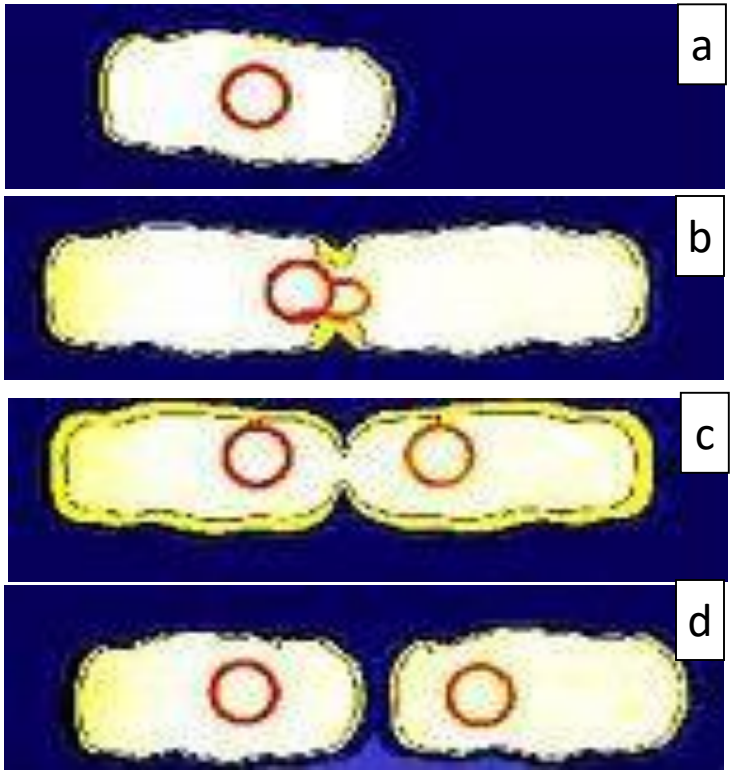
Bacteria multiply by binary fission, the process in which a parent cell splits into two daughter cells with approximately equal size.

a. Bacterial cell first can bee seen to enlarge or elongate.

b. Then followed by the formation of transverse membrane and new cell wall.

c. The new membrane and cell wall grow inward from the outer layers.

d. The cell divided into two daughter cells.



Growth: single cell dynamics

•**Under optimal conditions, the average time required for a population of bacteria to double in number (for complete cell division) is called as generation time or doubling time.**

•The **generation time** for many common bacteria is 20-30 min, for a few of slow-growing bacteria such as Tuberculosis bacteria might be up to 18-20 h.

Growth: population cell dynamics

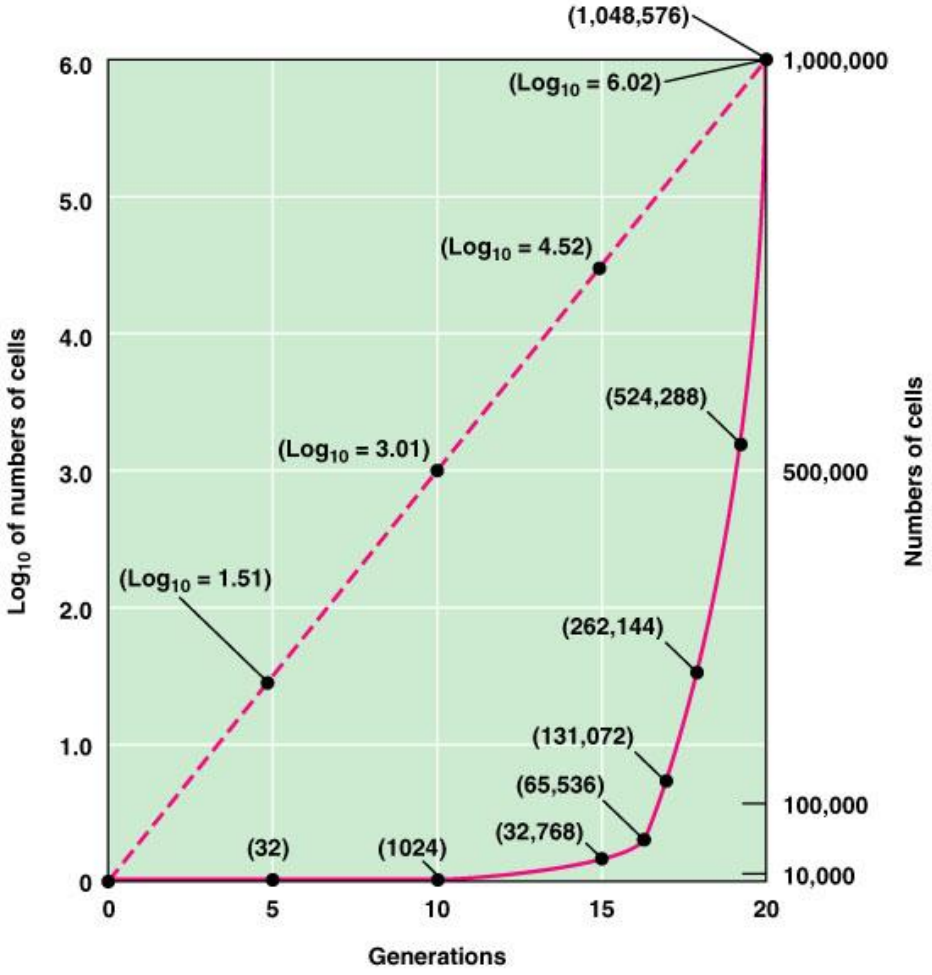
•When microorganisms are grown, due to some factors such as nutrient limitation and waste accumulation, growth rate cannot maintain for a long time.

•If a liquid medium is incubated with microbial cells, and the number of viable bacterial cells per milliliter is measured and plotted, we can obtain a curve that called as growth curve.

•Normally characterized by the phases lag, log (or exponential) growth, stationary growth, and death.

Generation Number	Number of Cells	Log_{10} of Number of Cells
0	1	0
5 (2^5) =	32	1.51
10 (2^{10}) =	1,024	3.01
15 (2^{15}) =	32,768	4.52
16 (2^{16}) =	65,536	4.82
17 (2^{17}) =	131,072	5.12
18 (2^{18}) =	262,144	5.42
19 (2^{19}) =	524,288	5.72
20 (2^{20}) =	1,048,576	6.02

(b)



How to Graph Bacterial Growth (i)

(I) Measuring the numbers of bacteria.

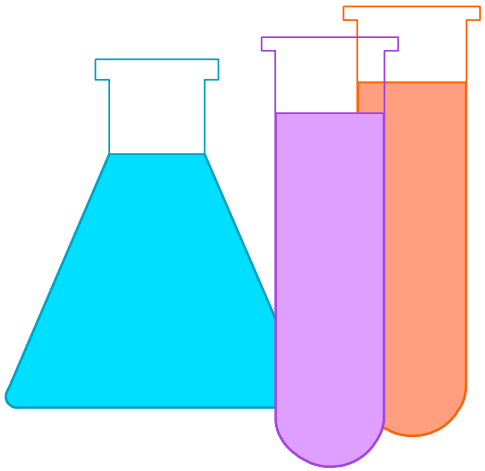
Common methods include (cell concentration):

a) Turbidity (the turbidity or Optical density which is the measure of the amount of light absorbed by a bacterial suspension): to measure the total bacteria (live and dead) in liquid cultures. This is usually quantitated with a spectrophotometer, the absorption wavelength at 600 nm will be measured.

b) Colony counting method: that means counting the colony numbers on a medium plate after inoculated with a known volume of bacterial liquid culture.

Measuring total bacteria (live + dead) in liquid culture——Turbidity (Cloudiness)

The cloudiness of a liquid media caused by bacteria growth that are generally invisible to the naked eye, similar to smoke in air.



Estimating Bacterial Numbers by Indirect Methods

- Turbidity

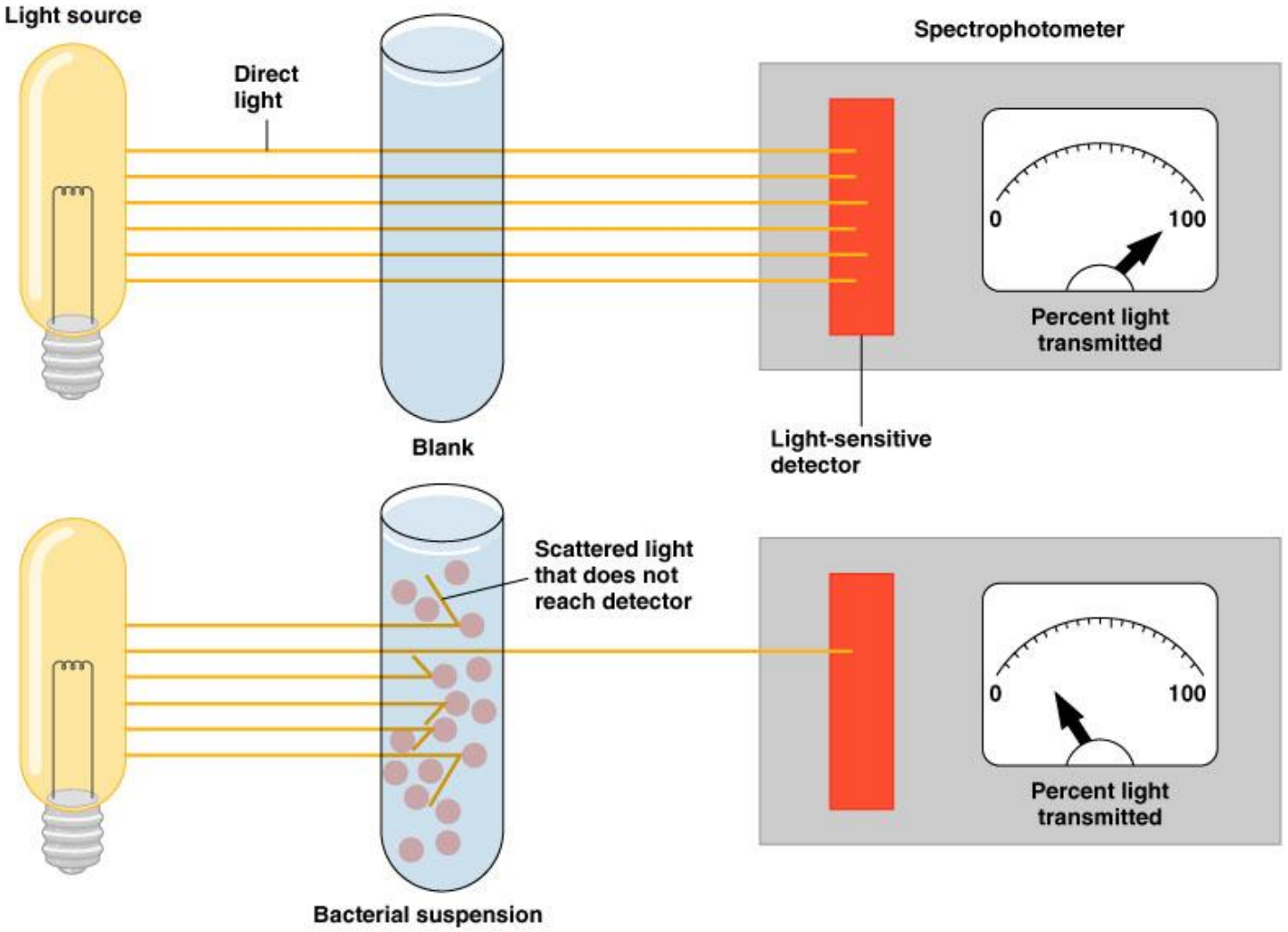
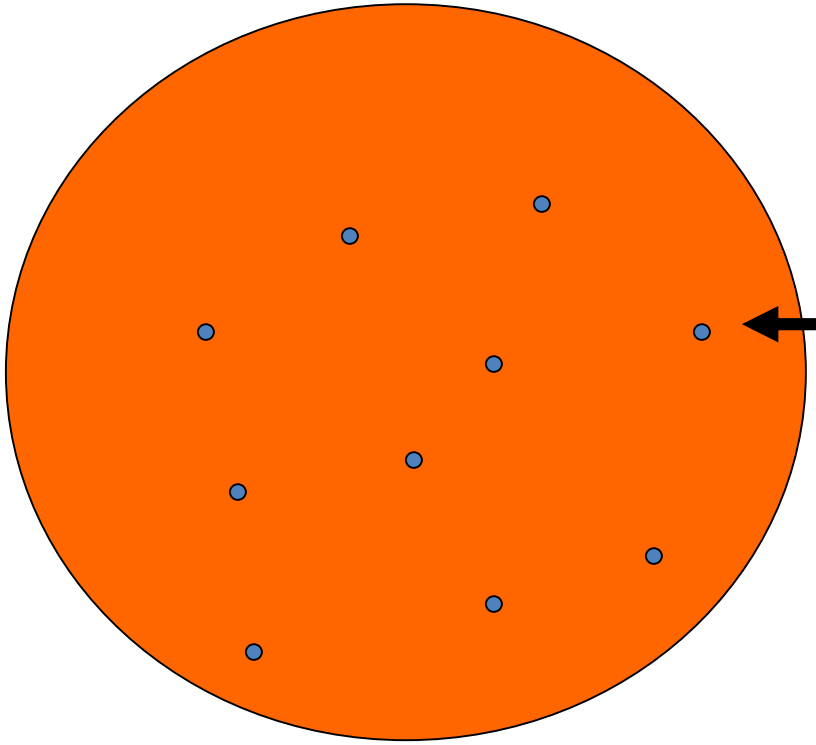


Figure 620

Measuring viable bacteria

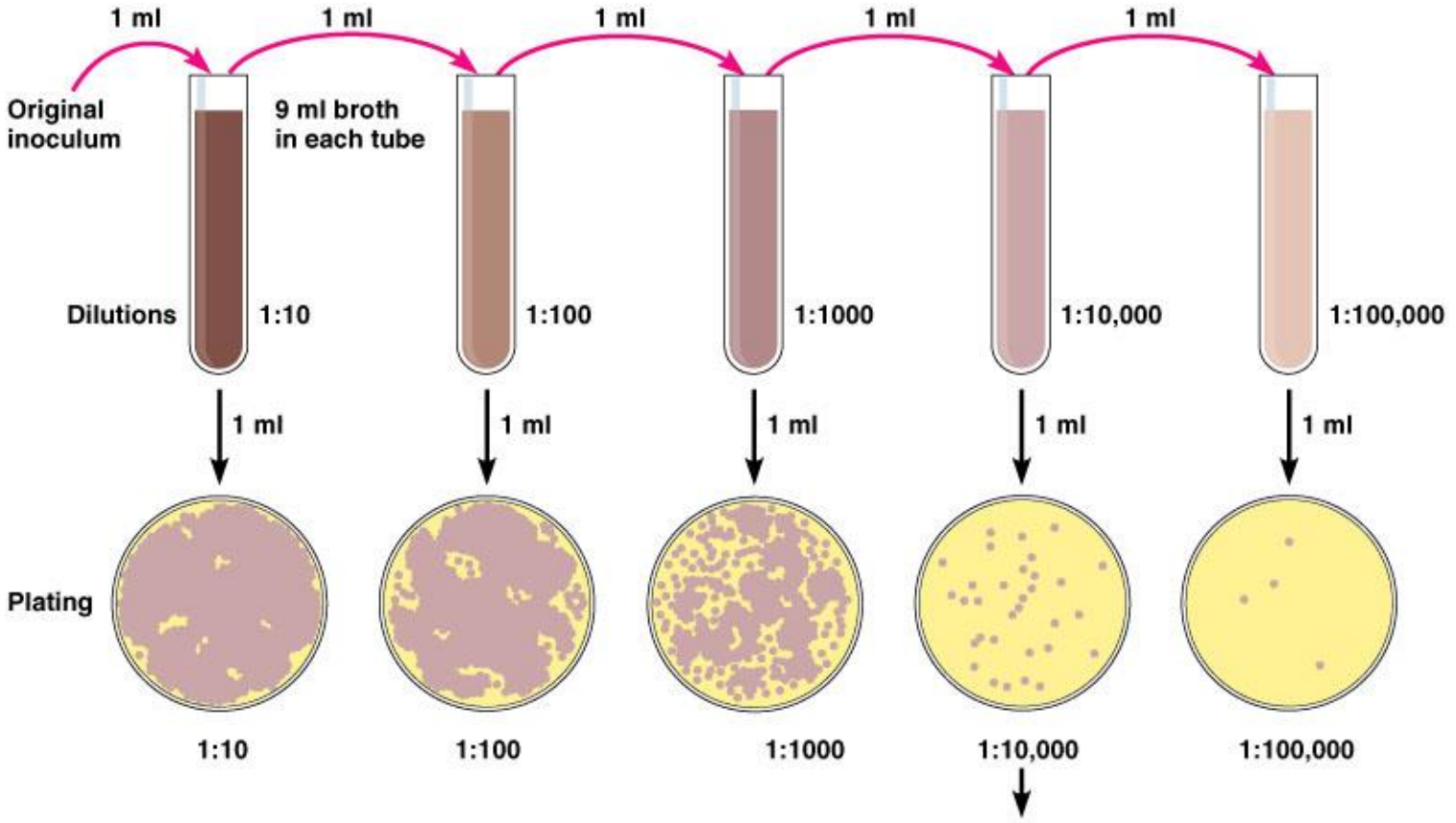


colony

A visible cluster of bacteria growing on the surface of or within a solid medium, presumably cultured from a single cell

Plate Assays: Spread Plate or Pour Plate Methods

- After incubation, count colonies on plates that have 30-300 colonies (CFUs)



The dilution in a particular tube = ml of fluid added to tube/total volume after addition; e.g. 1ml/(9ml + 1ml) = 1/10 = 10^{-1}

Calculation: Number of colonies on plate × reciprocal of dilution of sample = number of bacteria/ml
(For example, if 32 colonies are on a plate of $^{1}/_{10,000}$ dilution, then the count is 32 × 10,000 = 320,000/ml in sample.)

Figure 6.15

c- Biomass density: measured directly by determining the dry weight of a given culture or indirectly by measuring cellular component such as proteins.

An E. coli cell has a dry mass of about 7.0×10^{-19} mg.

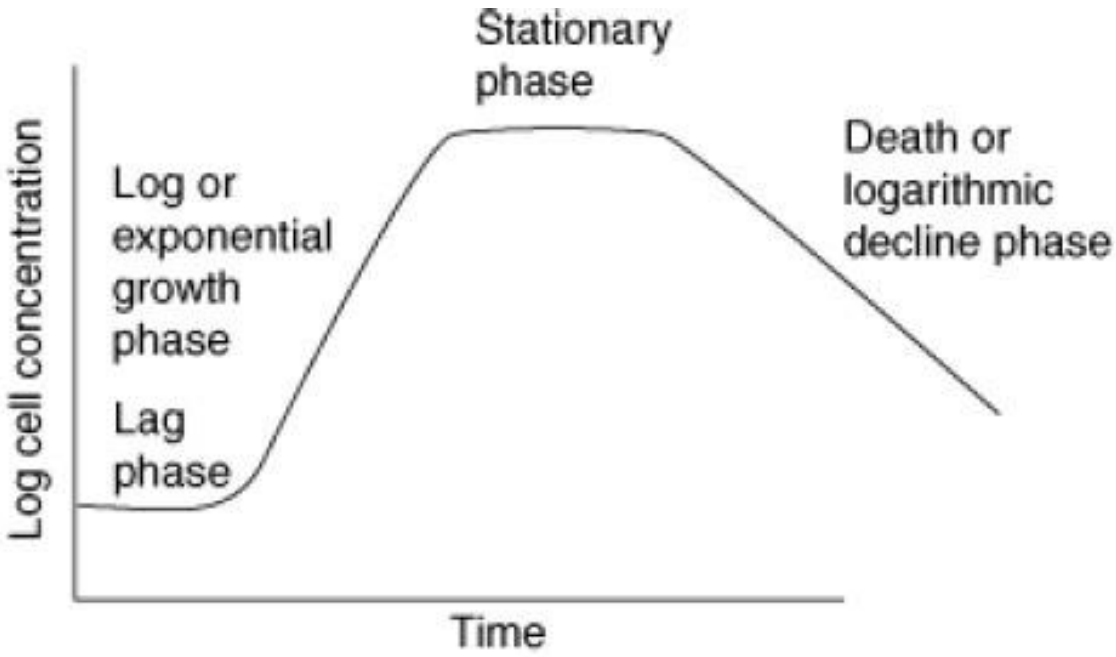
A 1 ml sample with a dry mass of 2 mg therefore has:

2 mg/ml x 1 cell/7×10^{-19} mg

$= 2.8 \times 10^{20}$ cells/ml

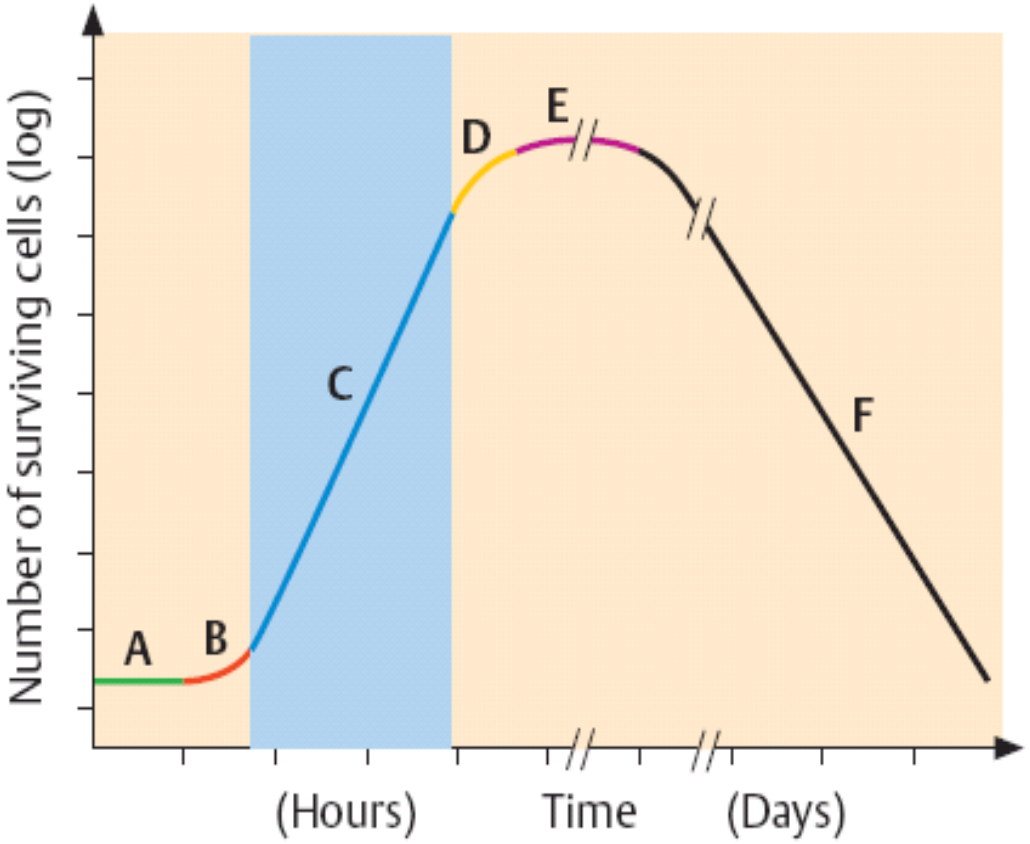
How to Graph Bacterial Growth (ii)

(II) Plotting the log of turbidity or number of living cells *versus* time is referred to as the growth curve (four or six phases):



Growth Curve (four phases)

Growth curve (six phases)



A: lag phase,
B: acceleration phase,
C: log (exponential) phase,
D: deceleration phase,
E: stationary phase,
F: death phase

Y-axis presents the log number of living cells
X-axis presents the period of time (usually in hours)
The curve can be divided into six phases represented by the letters A-F

What are the characteristics of bacteria in each phase?

Growth curve (four phases)

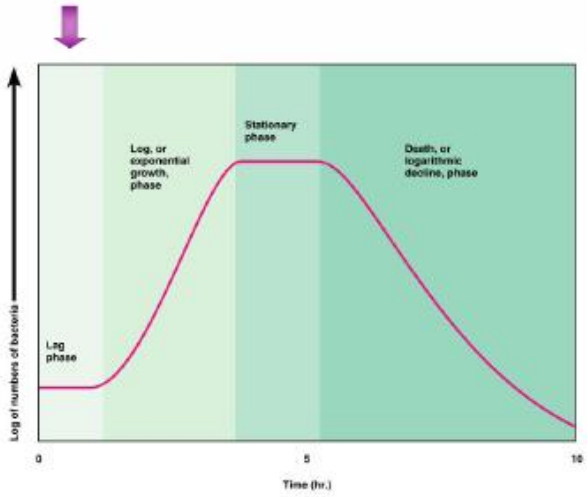
- **The Lag Phase (A and B):**

Bacteria are becoming "acclimated" with zero growth rate, to the new environmental conditions (pH, temperature, nutrients, etc.) (A).

Enzymes and intermediates are formed and accumulate until they are present in concentrations that are permit growth (B). Thus during this phase there is an increasing growth rate.

An increase in bacterial mass per unit of volume, but no increase in cell count.

The metabolism of the bacteria adapts to the conditions of the nutrient medium.



Growth curve (four phases)

- **The Exponential/log Phase (C):**

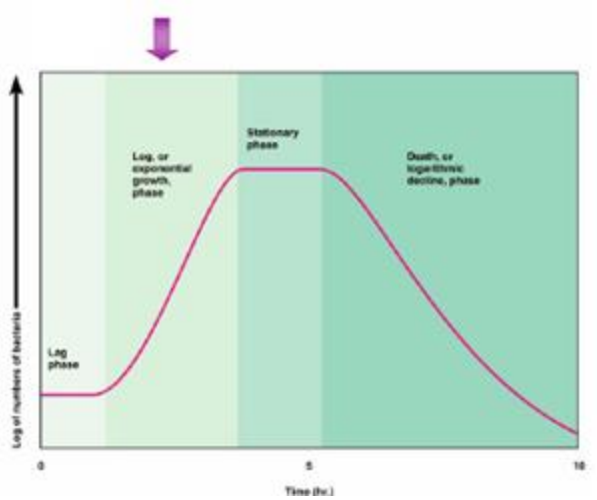
Conditions are optimal for growth.

The living bacteria population increases rapidly with time at an exponential growth in numbers, and the growth rate increasing with time.

The number of new bacteria appearing per unit time is proportional to the present population. If growth is not limited, doubling will continue at a constant rate so both the number of cells and the rate of population increase doubles with each consecutive time period.

The bacteria are suitable for biochemical and morphological identification,

The bacteria are suitable to use for drug sensitivity test

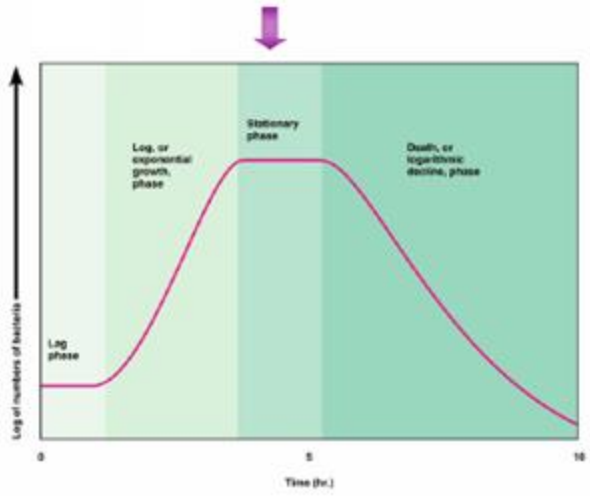


Growth curve (four phases)

- **The Maximum Stationary Phase (D and E):**

With the exhaustion of nutrients and accumulation of metabolic wastes, the growth rate has slowed to the point where the growth rate equals the death rate (D). Effectively, there is no net growth in the living bacteria population (E).

The bacteria produce spores, toxins (e.g. exotoxin) and antibiotics.

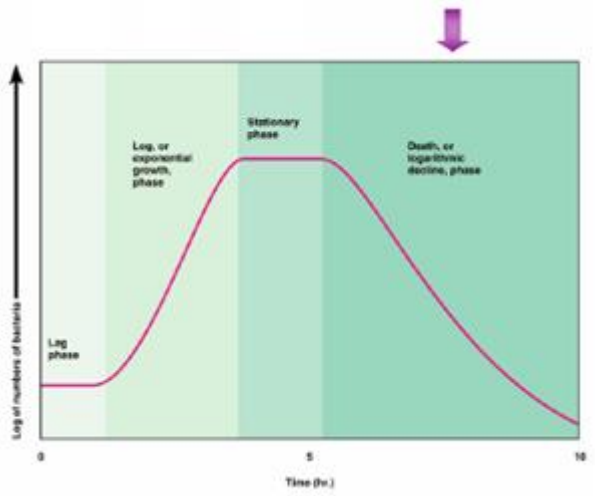


Growth curve (four phases)

- **The Decline Phase (F):**

The living bacteria population decreases with time, due to a lack of nutrients and toxic metabolic by-products.

In many cases (but not always) the bacteria autolyse (during the death phase) and the turbidity decreases with negative growth rate.



Brucella & klebsiella

By Dr. Zainab Al-Hakeem
By
Immunologist in microbiology

The main species of Klebsiella are:-

Klebsiella pneumonia

Klebsiella ozaenae

Klebsiella rhinoscleromatis

Klebsiella cloaca

- It is found in the mucosa of upper respiratory tract, intestine and genitourinary tract.
- It is gram negative, rod shape larger in size than *E. coli* it is non-motile, capsulated, growing on ordinary media forming large mucoid colonies of varying degree of stickiness,
- ferment carbohydrate forming acid& gas.
- Most species are pathogenic and classification depends on biochemical reactions which vary depend on capsular type.

Klebsiella pneumonia

It ferment sugar glucose, lactose, mannitol with production of acid and gas, indole M.R. negative VP citrate positive, hydrolysis of urea positive means urease test positive .

Cauese lobar pneumonia sinusitis, otitis media , meningitis serotypes 1,2,3 responsible for pneumonia .

Klebsiella ozaenae

It causes foul smelling nasal discharge (ozaena) Biochemical reaction are variable, it belong to capsules types 3,4,5,6.

Other strains cause granulomatous lesion of nose in Mediterranean countries.

Klebsiella rhinoscleromatis

Cause this disease **rhinoscleroma**. Organism are seen intracellular in lesion, it belong to capsular type 3.

Other strains of *klebsiella* named *klebsiella aerogenes* usually present in human intestine and isolated from faces in small number than *E. coli* so many survive longer out of intestine.

Klebsiella cloaca it differs in motility (+ve), liquefied gelatin (+ve) and not always capsulated.

Edwardsiella

It is non capsulted, motile bacilli with weak fermentation of sugar (glucose, maltose), it forms indole, H_2S and utilizes citrate .

Edwardsiell atarda is intestinal flora of snake, it also been isolated from human dirarrheic faces, however, its pathological rule is not known.

Cetrobacter

It occurs as intestinal commensal in man, motile, utilizes citrate, grows in KCN, produce H2S and may ferment lactose. It has two species:-

- *Citrobacter freundii*
- *Citrobacter intermedius* (H_2S negative).

It has been isolated from enteric fever cases, it may cause urinary tract infection, infection of gall bladder and meningitis …etc.

Enterobacter

It is motile, non capsulted, lactose fermenting, indole and M.R. negative,VP and citrate positive, it liquefied gelatin. There are 2 species:-

- *Enterobacter cloacae*
- *Enterobacter aerogenes* (found in human and animal faces

Soil …ect).

Hafina

It is also intestinal commensal; it is non-capsulated, motile, non-lactose fermenter, indole and M.R. negative, VP and citrate positive. Only one species is Known *Hafna alei*.

Serratia

It forms pink red or magenta non diffusible pigment, i.e. prodigiosin . Only one species is recognized *Serratia marcesens*.

Its motile non-capsulated, H2S -, VP +, citrate +,MR >70% -

It has been isolated from cases of meningitis, endocarditis, septicemia and respiratory infection. It may be an opportunist pathogen infecting debilitated patients of hospitals.

Voges–Proskauer test:

Positive Result:

Glucose ------Glucose Metabolism-------> Pyruvic Acid.

Pyruvic acid ----------> Acetoin.

Acetoin + added alpha-naphthol + added KOH = red color.

Negative Result:

Glucose ------Glucose Metabolism-------> Pyruvic Acid.

Pyruvic acid ----------> No Acetoin.

No acetoin + added alpha-naphthol + added KOH = copper color.

The Brucella:

- The brucella is obligate parasites of animals and humans and is characteristically located intracellularly.
- They are relatively inactive metabolically.
- *Brucella melitensis* typically infects goats; *Brucella suis*, swine; *Brucella abortus*, cattle; *and Brucella canis*, dogs.
- Other species are found only in animals.
- The disease in humans, brucellosis (undulant fever, Malta fever), is characterized by an acute bacteremic phase followed by a chronic stage that may extend over many years and may involve many tissues.

Growth Characteristics:

- Brucellae are adapted to an intracellular habitat, and their nutritional requirements are complex.
- Some strains have been cultivated on defined media containing amino acids, vitamins, salts, and glucose. Fresh specimens from animal or human sources are usually inoculated on trypticase-soy agar or blood culture media.

Variation

- The typical virulent organism forms a smooth, transparent colony; upon culture, it tends to change to the rough form, which is avirulent.
- The serum of susceptible animals contains a globulin and a lipoprotein that suppresses growth of non smooth, avirulent types and favors the growth of virulent types (smooth).

- Differentiation among Brucella species or biovars is made possible by their characteristic sensitivity to dyes and their production of H_2S.

The common routes of infection in humans are

- the intestinal tract (ingestion of infected milk),
- Mucous membranes (droplets), and
- Skin (contact with infected tissues of animals).

Brucellosis – Pathogenesis

Virulence factors

- Survival & replication inside macrophage
  - inhibition of the phagosome-lysosome fusion
  - prevention of toxic enzymes release from intracellular granule (catalase & SOD inactivate H_2O_2 & superoxide)
- Endotoxin

Phagocytised bacteria multiply in macrophages

⬇

Carried to liver, spleen, bone marrow, lymph nodes, kidneys

⬇

Multiply in cells of the reticuloendothelial system

⬇

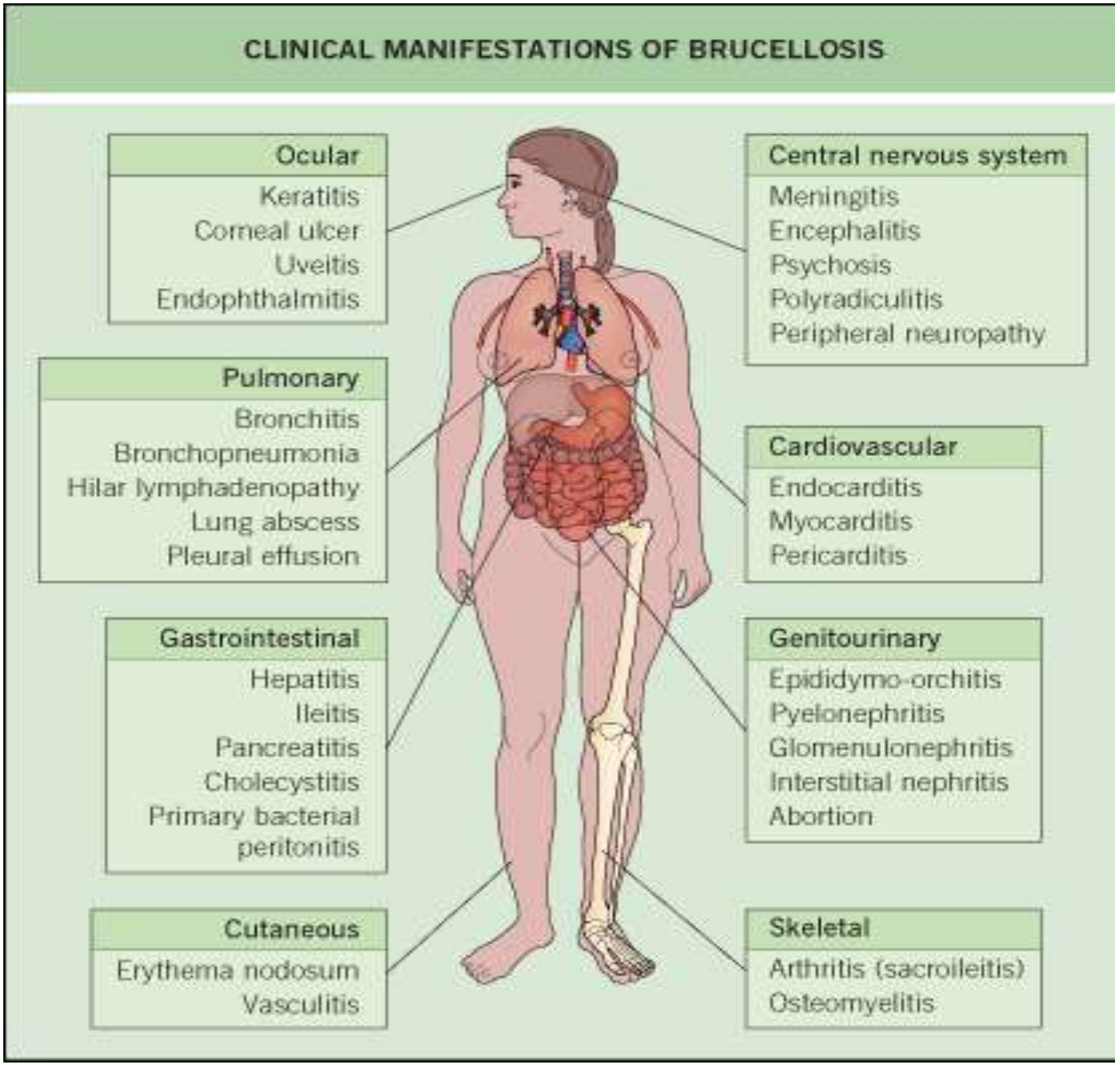
formation of small granulomas & release of bacteria in systemic circulation ➡ septicemia

Brucellosis – Clinical presentation

- Starts with nonspecific flu-like symptoms
- Night sweats and cycling pattern of nocturnal fever (undulant fever) – last for weeks to months
- Advance diseases
  - gastrointestinal (70% of patient), respiratory, cutaneous, neurologic or cardiovascular manifestations tract symptoms
  - osteolytic lesions or joint effusion
- Becomes a chronic illness with also body aches, headache, anorexia, weight loss and depression

In the natural host

- Mostly asymptomatic
- Affects organs rich in the sugar erythritol (breast, uterus, epididymis, etc.)
- Causes abortion, sterility and decreased milk production


CLINICAL MANIFESTATIONS OF BRUCELLOSIS
Ocular
Keratitis
Corneal ulcer
Uveitis
Endophthalmitis
Pulmonary
Bronchitis
Bronchopneumonia
Hilar lymphadenopathy
Lung abscess
Pleural effusion
Gastrointestinal
Hepatitis
Ileitis
Pancreatitis
Cholecystitis
Primary bacterial peritonitis
Cutaneous
Erythema nodosum
Vasculitis
Central nervous system
Meningitis
Encephalitis
Psychosis
Polyradiculitis
Peripheral neuropathy
Cardiovascular
Endocarditis
Myocarditis
Pericarditis
Genitourinary
Epididymo-orchitis
Pyelonephritis
Glomerulonephritis
Interstitial nephritis
Abortion
Skeletal
Arthritis (sacroileitis)
Osteomyelitis

Diagnostic Laboratory Tests:

Specimens:

❖Blood should be taken for culture

❖biopsy material for culture (lymph nodes, bone, etc),

❖serum for serologic tests

culture

The medium is highly enriched and—in reduced form—is used primarily in cultures for anaerobic bacteria. In oxygenated form, the medium grows Brucella species bacteria very well.

Media:

trypticase-soy medium with or without 5% sheep blood, brain heart infusion medium, and chocolate agar, Blood culture media , Liquid medium used to culture Mycobacterium tuberculosis also supports the growth of at least some strains.

❖All cultures should be incubated in 8–10% CO2at 35–37°C and should be observed for 3 weeks before being discarded as negative

❖Negative cultures for Brucella do not exclude the disease because brucella can be cultivated from patients only during the acute phase of the illness or during recurrence of activity.

Agglutination test—

❖To be reliable, serum agglutination tests must be performed with standardized heat-killed, phenolized, smooth Brucella antigens.

❖IgG agglutinin titers above 1:80 indicate active infection. (rose bengal test).

Serology

- IgM antibody levels rise during the first week of acute illness, peak at 3 months, and may persist during chronic disease.
- Even with appropriate antibiotic therapy, high IgM levels may persist for up to 2 years in a small percentage of patients.
- IgG antibody levels rise about 3 weeks after onset of acute disease, peak at 6–8 weeks, and remain high during chronic disease.
- IgA levels parallel the IgG levels. The usual serologic tests may fail to detect infection with B canis. (detected by ELISA)

Blocking antibodies—

Brucella antibody

Positive titer ≥1:80

↑≥ 4-fold in serum specimens obtained >2 weeks apart.

negative

absence of infection by *Brucella* infection

False negative

- *B canis* infection
- Technical errors

positive

- Brucella infection (except B canis)

False positive in:

- infections with *Francisella tularensis, Yersinia enterocolitica*, salmonella, Rocky Mountain spotted fever; vaccinations for cholera
- Technical errors

Brucellosis – Diagnosis

- Microscopy – insensitive because small size and intracellular
- Blood culture
  - slow growth; non-hemolytic colonies
- Serologic tests
  - most common
  - increase in antibody titer = current disease

Brucellosis – Treatment & Prevention

- Treatment
  - Combination of doxycycline + rifampin
  - Mortality is low (<2%), and is usually associated with endocarditis
- Prevention
  - Minimize occupational exposure
  - Pasteurization of dairy products
  - Control of Brucellosis in animals (immunization, eradication of infected stock)
  - No human vaccine

Campylobacter

Campylobacter (meaning 'twisted bacteria') is a genus of bacteria that are Gram-negative, spiral, and microaerophilic. Motile, with either unipolar or bipolar flagella, the organisms have a characteristic spiral/corkscrew appearance and are oxidase-positive. They are adapted to colonize the surface of the mucous membranes of the alimentary and reproductive tracts. This adaptation is reflected in their morphology.

Species:

•*Campylobacter jejuni*: one of the main causes of bacterial foodborne disease in many developed countries.

•*C. coli*: implicated in human disease.

•*C. fetus*: cause of spontaneous abortions in cattle and sheep, as well as an opportunistic pathogen in humans.

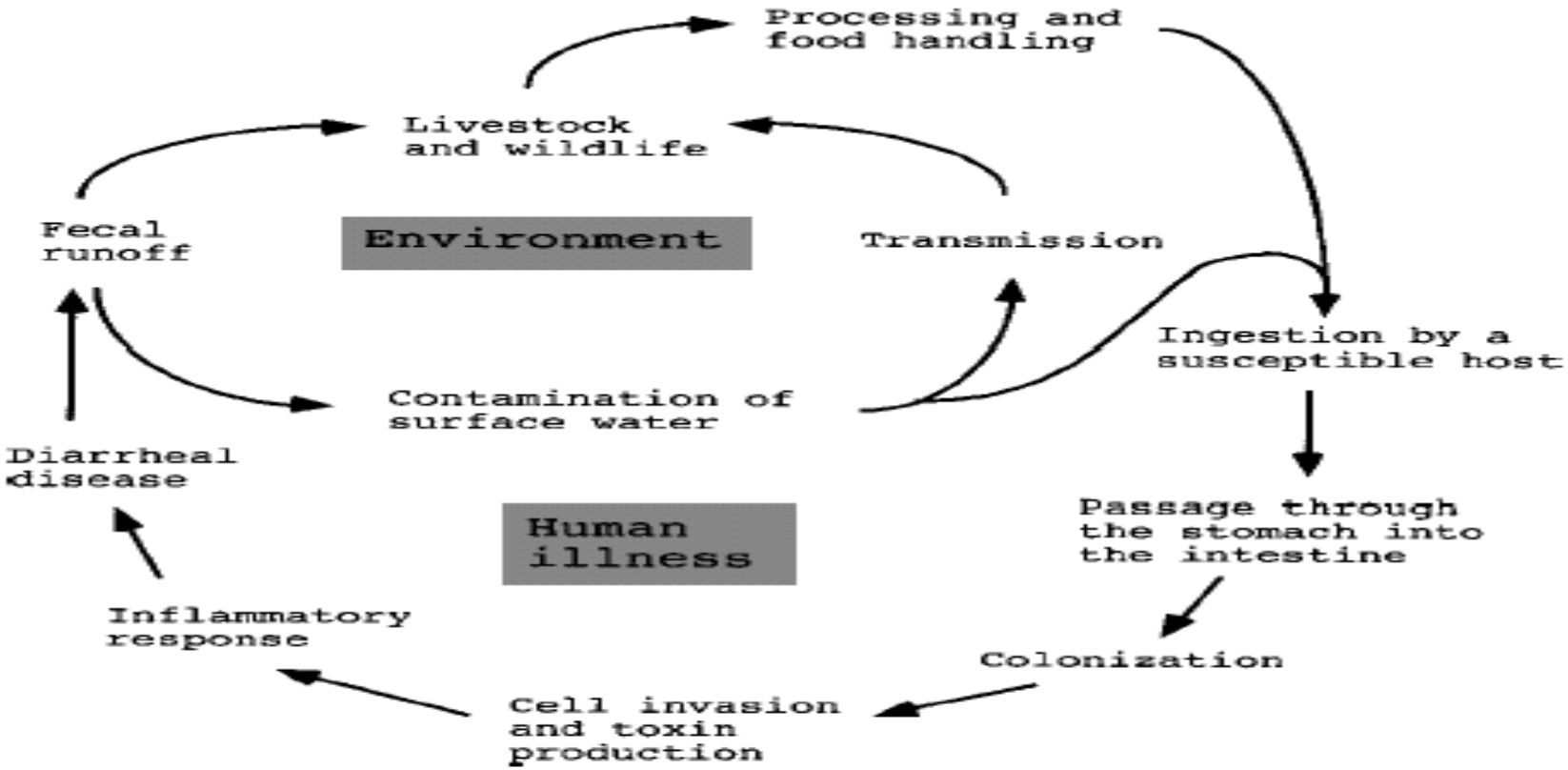


Figure 1. *C. jejuni* infections are commonly acquired by handling and consuming undercooked chicken, and drinking unpasteurized milk and polluted water. Human illness with *C. jejuni* ranges from mild to severe diarrheal disease, the latter of which is characterized by the presence of blood and leukocytes in stool specimens.

Toxin: Most strains of *C jejuni* produce a toxin (cytolethal distending toxin) that hinders the cells from dividing and activating the immune system. This helps the bacteria to evade the immune system and survive for a limited time in the cells.

Treatment:

Standard treatment is now azithromycin. Quinolone antibiotics such as ciprofloxacin or levofloxacin are no longer as effective due to resistance.

Diagnosis:

Specimen: stool

•Direct examination: Fecal leukocytes should be present, and gram stain reviled gram-negative.

•Darting motility in hanging drop, oxidase positive, and catalase positive

•Stool culture: Colonies are moist, runny-looking and spreading, non hemolytic.

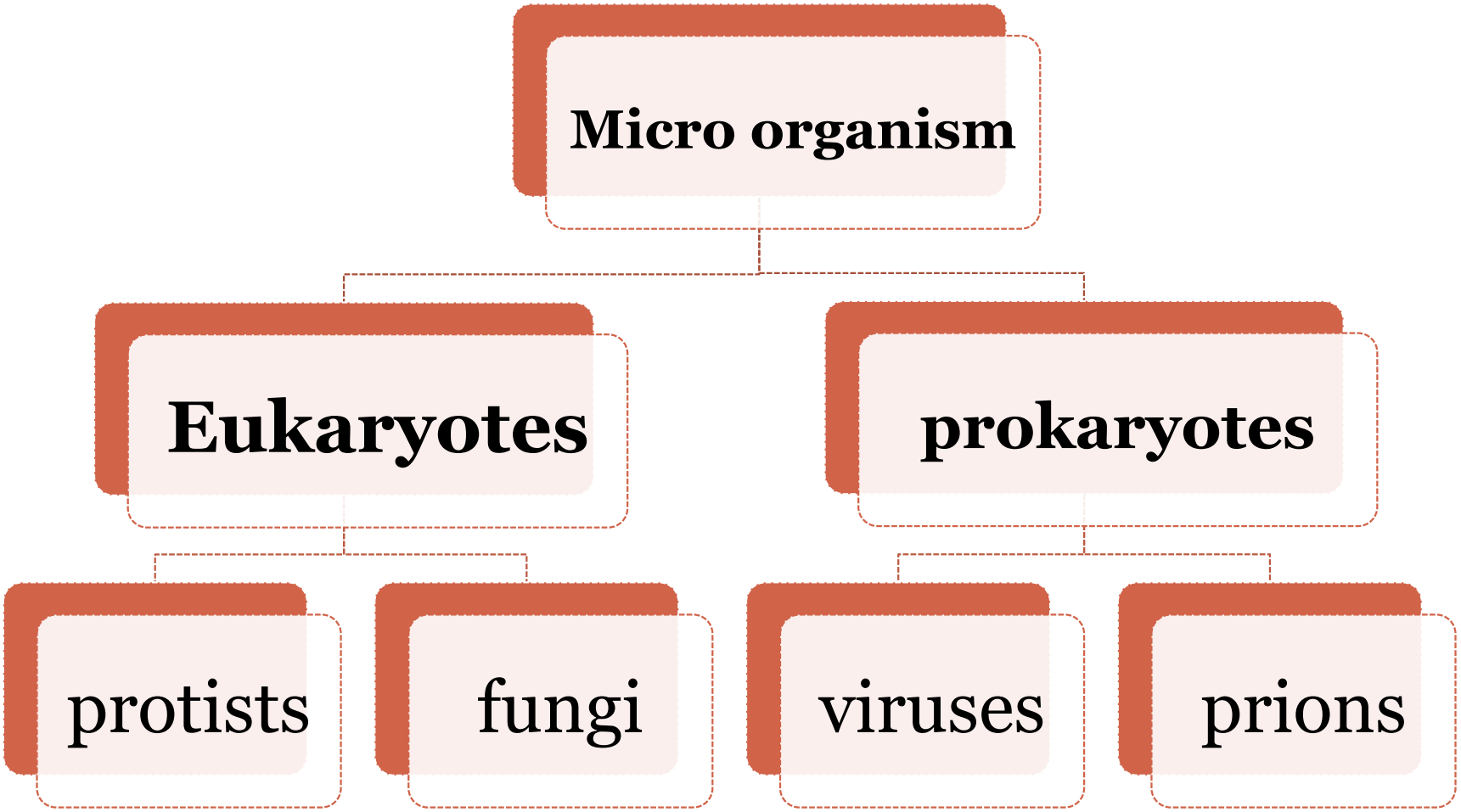
•Methods currently being developed to detect the presence of campylobacter organisms include antigen testing via an EIA or PCR.

Introduction to Microbiology

By Dr. Zainab Al-Hakeem

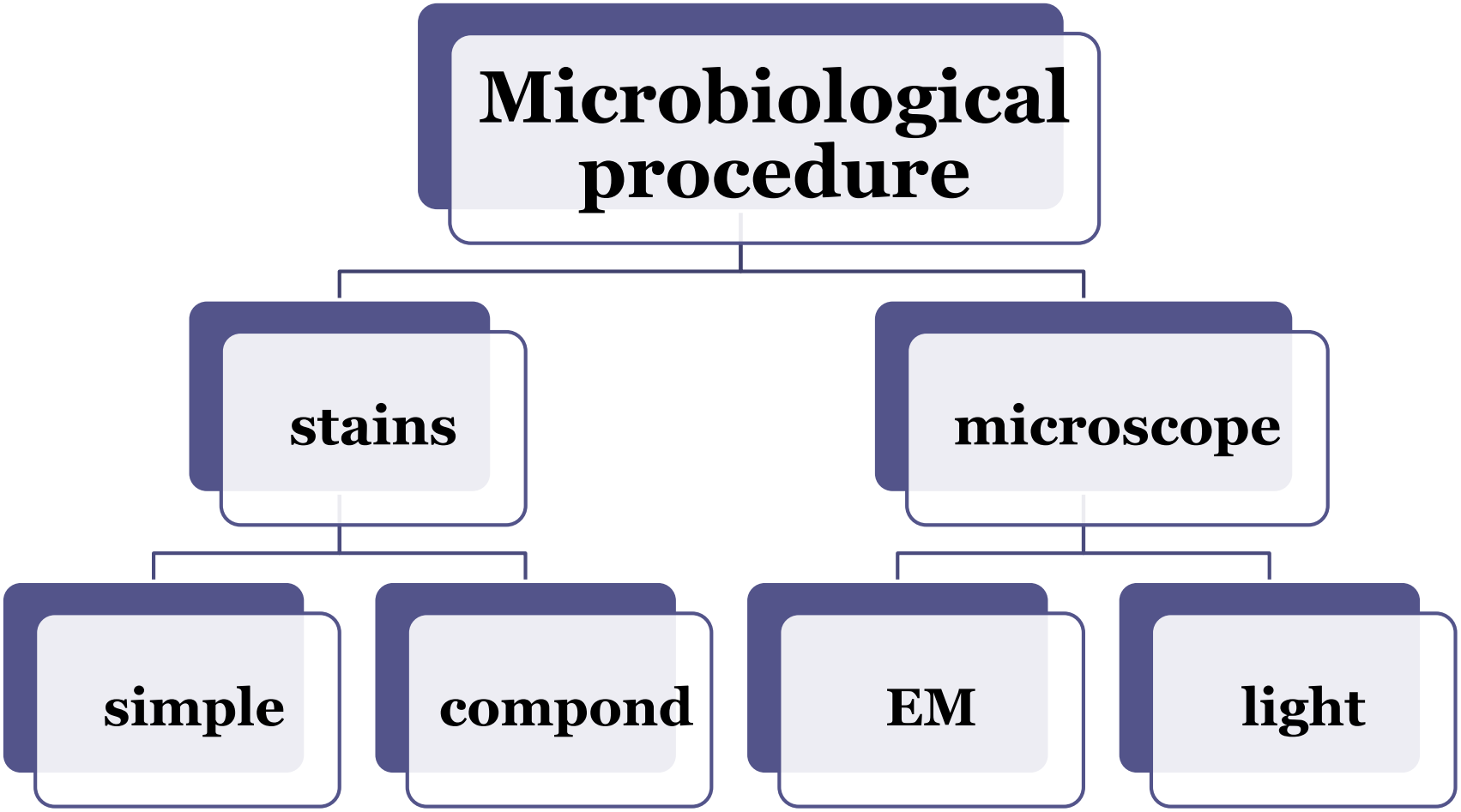
Immunologist in microbiology

Microbiology (from Greekmīkros, "small” bios, "life";-logia) is the study of microscopic organisms, which are defined as any living organism that is either a single cell (unicellular), a cell cluster, or has no cells at all (acellular),which, must be viewed with the aid of a microscope or electron microscope


Micro organism
Eukaryotes
prokaryotes
protists
fungi
viruses
prions

Microbiology is a broad term which includes

- virology,
- mycology,
- Parasitology,
- bacteriology,
- immunology and other branches. A microbiologist is a specialist in microbiology and these related topics.


Microbiological procedure
stains
microscope
simple
compond
EM
light

Historical view:

•Ancient (before the discovery of the microscope): The existence of microorganisms was hypothesized for many centuries before their actual discovery.

•existence of unseen microbiological creatures living in earth, water, air and fire. Jain scriptures also describe nigodas which are sub-microscopic creatures living in large clusters and having a very short life and are said to pervade each and every part of the universe, even in tissues of plants and flesh of animals.

•Modern (after the microscope invention):

•1660’s Robert Hooke observed microorganisms for the first time with a microscope and coined the term “cell”. He observed the fruiting bodies of molds.

•1632-1723 Anton van Leeuwenhoek a Dutch scientist, credited with having observed the first bacteria. One of his books contains a chapter in Latin, which reads in translation – ‘Concerning the wonderful structure of things in nature, investigated by Microscope.

•Lazzaro Spallanzani (1729–1799) found that boiling broth would sterilise it and kill any microorganisms in it.

•**1828-1898 Ferdinand Cohn developed the first classification scheme based on bacteria shape. Cohn detailed and described the life cycle of Bacillus**

•**1822-1895 Louis Pasteur Defined pasteurization to prevent spoilage of food by bacteria, develop vaccines and disproved "Spontaneous Generation". He defined "Germ Theory" and demonstrated that germs were responsible for disease.**

•**1843-1910 Robert Koch identified anthrax and developed agar growth medium. Koch's postulates were a systematic method to establish the microbial cause of disease.**

Branches of microbiology:

The branches of microbiology can be classified into pure and applied sciences.

Pure microbiology

Taxonomic arrangement

- **Bacteriology: The study of bacteria.**
- **Mycology: The study of fungi.**
- **Protozoology: The study of protozoa.**
- **Phycology(or algology): The study of algae.**
- **Parasitology: The study of parasites.**
- **Immunology: The study of the immune system.**
- **Virology: The study of viruses.**
- **Nematology:The study of the nematodes**

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Integrative arrangement

- **Microbial cytology: The study of microscopic and submicroscopic details of microorganisms.**
- **Microbial physiology: The study of how the microbial cell functions biochemically. Includes the study of microbial growth, microbial metabolism and microbial cell structure.**
- **Microbial ecology: The relationship between microorganisms and their environment.**
- **Microbial genetics: The study of how genes are organized and regulated in microbes in relation to their cellular functions. Closely related to the field of molecular biology.**
- **Cellular microbiology: A discipline bridging microbiology and cell biology.**

•**Evolutionary microbiology: The study of the evolution of microbes. This field can be subdivided into:**

•**Microbial taxonomy: The naming and classification of microorganisms.**

•**Microbial systematics: The study of the diversity and genetic relationship of microorganisms.**

•**Generation microbiology: The study of those microorganisms that have the same characters as their parents.**

•**Systems microbiology: A discipline bridging systems biology and microbiology.**

•**Molecular microbiology: The study of the molecular principles of the physiological processes in microorganisms.**

Other

- **Nano microbiology: The study of those microorganisms on nano level.**
- **Exomicrobiology (or Astro microbiology): The study of microorganisms in outer space.**
- **Weapon microbiology: The study of those microorganisms which are used in weapon industries.**

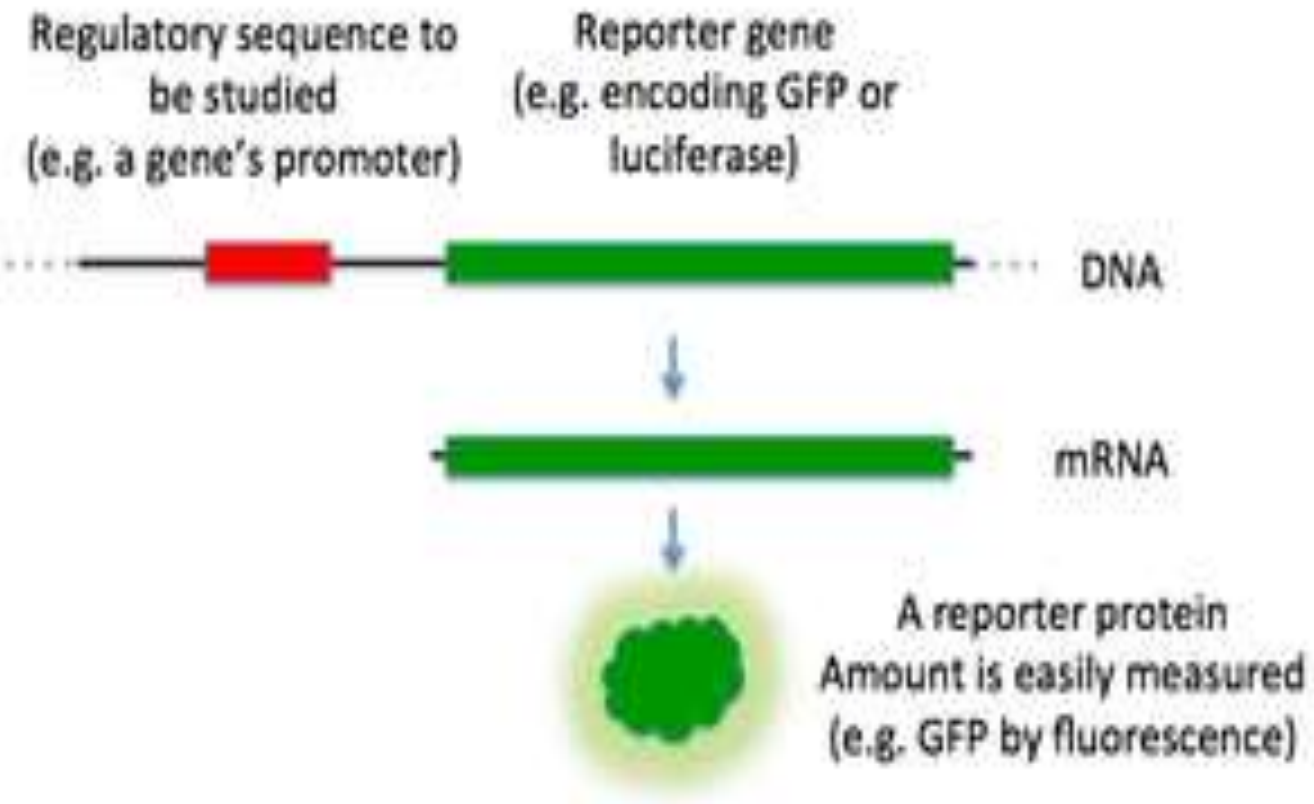
Applied microbiology

Medical microbiology	• The study of the pathogenic microbes and the role of microbes in human illness. Includes the study of microbial pathogenesis and epidemiology and is related to the study of disease pathology and immunology.
Pharmaceutical microbiology	• The study of microorganisms that are related to the production of antibiotics, enzymes, vitamins,vaccines, and other pharmaceutical products and that cause pharmaceutical contamination and spoil.
Industrial microbiology	The exploitation of microbes for use in industrial processes. • Examples include industrial fermentation and waste water treatment
Microbial biotechnology	• The manipulation of microorganisms at the genetic and molecular level to generate useful products.

Food microbiology and Dairy microbiology	• The study of microorganisms causing food spoilage and foodborne illness. Using microorganisms to produce foods, for example by fermentation.
Agricultural microbiology	• The study of agriculturally relevant microorganisms. This field can be further classified into the following: • Plant microbiology and Plant pathology
Soil microbiology:	• The study of those microorganisms that are found in soil.
Veterinary microbiology	• The study of the role microbes in veterinary medicine or animal taxonomy.

Benefits:

- Industrial fermentation (e.g. the production of alcohol (yeast), vinegar (Acetobacteraceae) and dairy products).
- Antibiotic production (the beta-lactam antibacterials, which include the penicillins (produced by fungi in the genus *Penicillium*), the cephalosporins
- Vehicles for cloning in more complex organisms such as plants.
- Biotechnological production of important enzymes such as Taq polymerase (*Thermis aquaticus)*, reporter genes for use in other genetic systems and novel molecular biology techniques such as the yeast two-hybrid system.



A diagram of a how a reporter gene is used to study a regulatory sequence.

❖Microorganisms are used for the biosynthesis of xanthan, alginate, cellulose.

❖Microorganisms are beneficial for microbial biodegradation or bioremediation of domestic, agricultural and industrial wastes and subsurface pollution in soils, sediments and marine environments.

❖There are also various claims concerning the contributions to human and animal health by consuming probiotics (bacteria potentially beneficial to the digestive system) and/or prebiotics

Growth requirements

By Dr. Zainab Al-Hakeem

Immunology| microbiology

Factors Influencing Bacterial Growth

1. Physical requirements

a. Temperature

Bacteria have a minimum, optimum, and maximum temperature for growth and can be divided into 3 groups based on their optimum growth temperature:

1. **Psychrophiles:** are cold-loving bacteria. Their optimum growth temperature is between -5C and 15C. They are usually found in the Arctic regions. Their importance lies in their ability to grow & cause spoilage

2. **Mesophiles:** are bacteria that grow best at moderate temperatures. Their optimum growth temperature is between 25C and 45C. Most bacteria are mesophilic and include common soil bacteria and bacteria that live in and on the body causing disease e.g. *Nessieria gonorrhea*.

Staphylococcus aureus

Staphylococcus aureus is prevalent among the human population, and in many cases, is carried by humans without ailment.

The Centers for Disease Control, for example, suggest that almost a third of the population carries staphylococcus aureus in their noses.

This bacteria, however, can cause staph infections, which can range from skin infections to blood poisoning, and can be significantly more serious when contracted in health care settings, such as a hospital.

While traditional staph infections are typically responsive to antibiotic treatment, methicillin-resistant Staphylococcus aureus, also known as MRSA, is remarkably resistantagainst many antibiotics, and thus, is incredibly hard to combat medically.

Salmonella

Though it can live without harm in a person's intestinal tract, salmonella is a frequent cause of food poisoning in humans. Introduced through contaminated water, contaminated foods, and sometimes, contact with animals, such as reptiles or birds, salmonella can infect the intestines, causing symptoms ranging from a fever and mild diarrhea to severe dehydration or blood poisoning.

Listeria monocytogenes

Another common mesophilic bacterium is listeria monocytogenes, which is most often circulated through contaminated food, such as uncooked meats or unpasteurized cheeses. Animals and humans can carry listeria, but it poses the greatest threat to those with weakened immune systems, such as pregnant women or the elderly. According to the Centers for Disease Control, listeria is the third leading cause of food poisoning-related deaths.

3. Thermophiles: or facultative thermophiles are heat-loving bacteria. Their optimum growth temperature is between 45C and 70C and are commonly found in hot springs. Their importance lies in their ability to produce spores and cause spoilage of canned foods.

4. Hyperthermophiles: are bacteria that grow at very high temperatures. Their optimum growth temperature is between 70C and 110C. They are usually members of the Archae and are found growing near hydrothermal vents at great depths in the ocean.

1. Thermophiles are adapted to temperatures above 60 degrees in a variety of ways. Often thermophiles have a high G + C content in their DNA such that the melting point of the DNA (the temperature at which the strands of the double helix separate) is at least as high as the organism's maximum T for growth.

2. The membrane fatty acids of thermophilic bacteria are highly saturated allowing their membranes to remain stable and functional at high temperatures. The membranes of hyperthermophiles, virtually all of which are Archaea, are not composed of fatty acids but of repeating subunits of the C5 compound, saturated, "isoprenoid"

3. The structural proteins (e.g. ribosomal proteins, transport proteins and enzymes are modified in a number of ways including dehydration and through slight changes in their primary structure, which accounts for their thermal stability.

Oxygen requirements

Microorganisms show a great deal of variation in their requirements for gaseous oxygen. Most can be placed in one of the following groups:

1. **Obligate aerobes (aerobic):** are organisms that grow only in the presence of oxygen. They obtain their energy through aerobic respiration.

- They require O2 as a hydrogen acceptor e.g. Pseudomonadaceae, Bacillus, nitrobacter,---.
- Aerobic bacteria use oxygen to break down pyruvic acid, releasing much more ATP than is produced during glycolysis during the process known as **aerobic respiration**.
- In addition, aerobic bacteria have enzymes such as superoxide dismutase capable of breaking down toxic forms of oxygen, such as superoxide free radicals, which are also formed by aerobic respiration.

TOXIC FORMS OF OXYGEN

- Singlet oxygen: O_2 boosted to a higher-energy state
- Superoxide free radicals: O_2^-

$$O_2^- + 2\,H^+ \xrightarrow{\text{superoxide dismutase}} H_2O_2 + O_2$$

- Peroxide anion: O_2^{2-}

$$2\,H_2O_2 \xrightarrow{\text{catalase}} 2\,H_2O + O_2$$

$$H_2O_2 + 2\,O^+ \xrightarrow{\text{peroxidase}} 2\,H_2O$$

- Hydroxyl radical (OH•)

- During aerobic respiration, enzymes remove electrons from the organic substrate and transfer them to the electron transport chain, which is located in the membrane of the mitochondrion.

- The electrons are transferred along a chain of electron carrier molecules.
- At the final transfer position, the electrons combine with atoms of oxygen—the final electron acceptor—which in turn combines with protons (H+) to produce water molecules.

- Energy, in the form of ATP, is also made here. Along the chain of electron carriers, protons that are pumped across the mitochondrial membrane re-enter the mitochondrion.

- This flow of electrons across the membrane fuels oxidative phosphorylation, the chemical reaction that adds a phosphate group to adenosine diphosphate (ADP) to produce ATP.

2. Microaerophiles: are organisms that require a low concentration of oxygen (2% to 10%) for growth, but higher concentrations are inhibitory. They obtain their energy through aerobic respiration.

3. Obligate anaerobes: are organisms that grow only in the absence of oxygen and, in fact, are often inhibited or killed by its presence.

❖They obtain their energy through anaerobic respiration or fermentation.

❖It is widely accepted that obligate (strict) anaerobes die in the presence of oxygen due to the absence of the enzymes **superoxide dismutase** and **catalase**, which would convert the lethal superoxide formed in their cells due to the presence of oxygen.

❖Electron acceptor is sulfate, nitrate, iron, manganese, mercury, and carbon monoxide

4. Aerotolerant anaerobes: like obligate anaerobes, cannot use oxygen to transform energy but can grow in its presence.

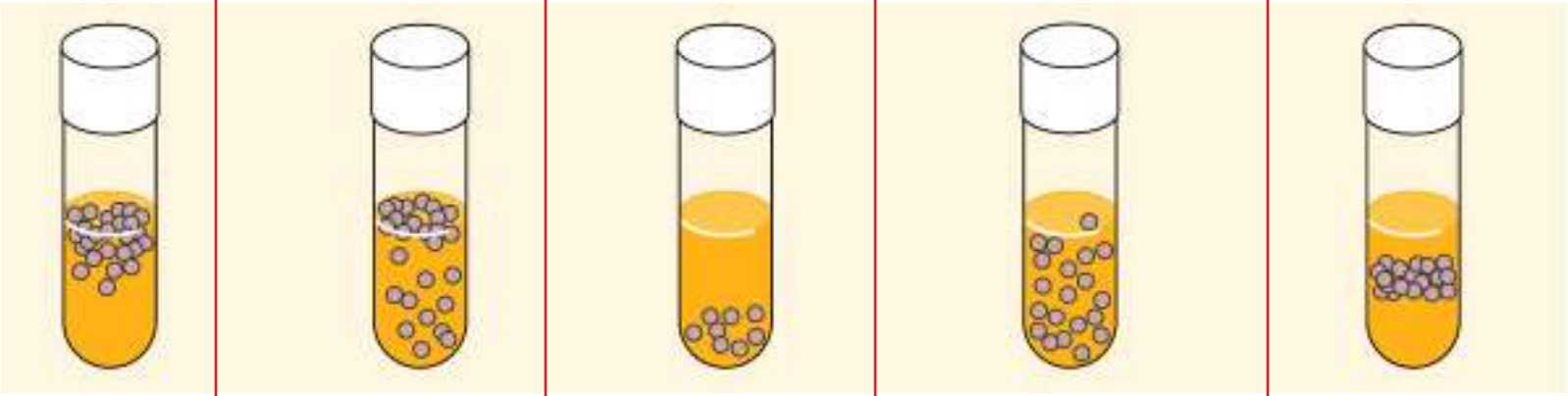
•They obtain energy only by fermentation and are known as obligate fermenters.

5. Facultative anaerobes: are organisms that grow with or without oxygen, but generally better with oxygen.

•They obtain their energy through aerobic respiration if oxygen is present, but use fermentation or anaerobic respiration if it is absent.

•Most bacteria are facultative anaerobes. E.g. *Vibrio*, *E. coli*, *Salmonella*, *Shigella*,----.

- Oxygen (O_2)

Obligate aerobes	Faultative anaerobes	Obligate anaerobes	Aerotolerant anaerobes	Microaerophiles
				

c. pH

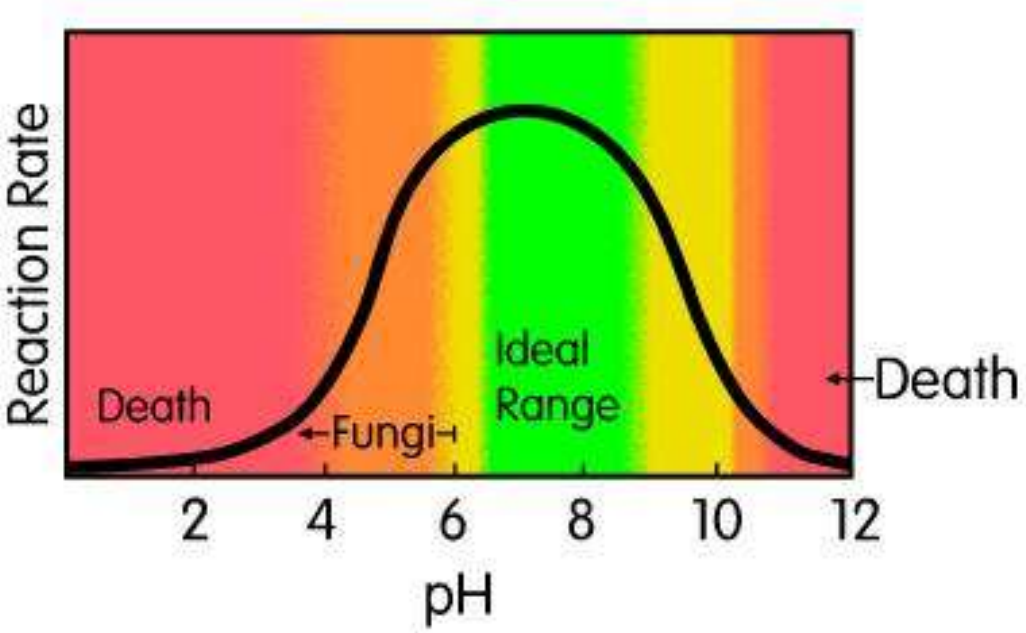
Microorganisms can be placed in one of the following groups based on their optimum pH requirements:

1. Neutrophiles grow best at a pH range of 5 to 8 (most pathogenic bacteria are within this group).

2. Acidophiles grow best at a pH below 5.5 e.g. *lactobacillus*.

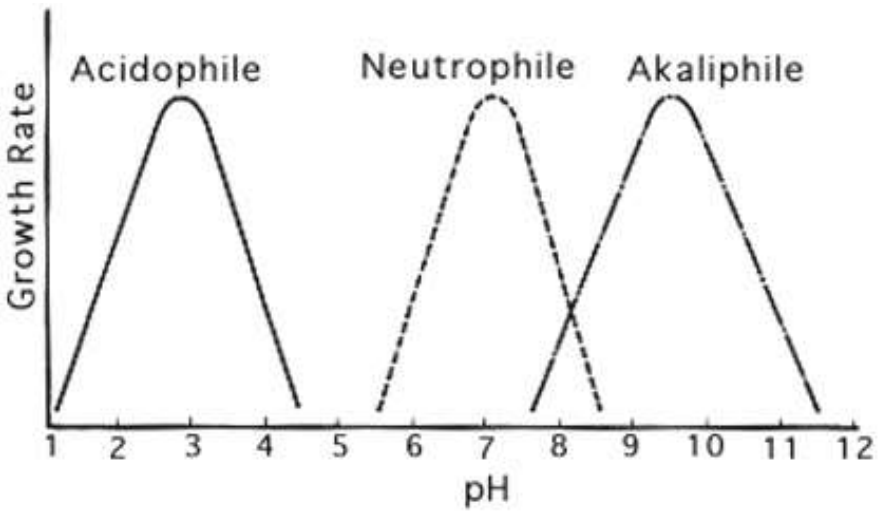
3. Alkaliphiles grow best at a pH above 8.5*Vibrio cholera*.

Physical Requirements: pH



Define pH mathematically
Know number range

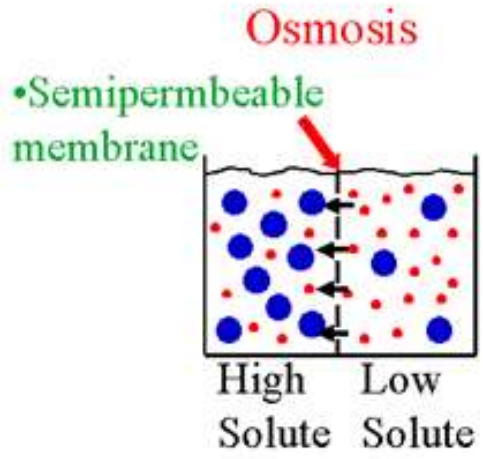
pH Groups

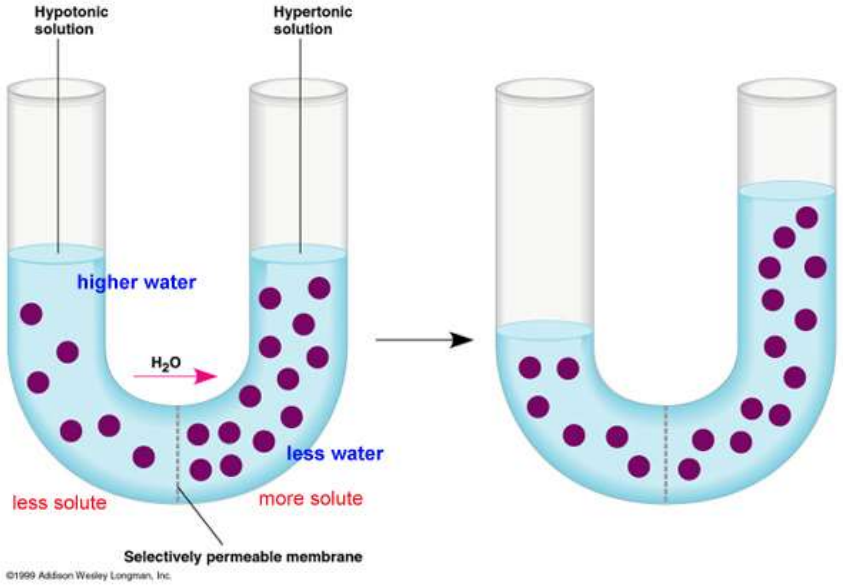


- Acidophile
  - *Bacillus acidocaldarius*
  - *Lactobacillus*
- Neutrophile
  - *E. coli*
  - *Staphylococcus aureus*
- Akaliphiles
  - *Streptococcus pneumoniae*
  - *Nitrobacter sp.*

Physical Effect of Water

- osmosis: is the diffusion of water across a membrane from an area of higher water concentration (lower solute concentration) to lower water concentration (higher solute concentration).
- Osmotic Pressure: is the pressure applied by a solution to prevent the inward flow of water across a semipermeable membrane.
  - Tonicity
    - Isotonic
    - Hypertonic (high solute con. outside)
    - Hypotonic (low = = = =)
  - Effects
  - Osmotolerant
- Hydrostatic Pressure
  - Barotolerant
  - Barophiles (deep ocean)





- Most bacteria require an isotonic environment or a hypotonic environment for optimum growth.

- Organisms that can grow at a relatively high salt concentration(up to10%) are said to be osmotolerant.

- Those that require relatively high salt concentrations for growth, like some of the Archea that require sodium chloride concentrations of 20 % or higher halophiles.

- Exposure of bacteria to such high concentration of salts cause protoplast shrinkage which occur more in G-ve bacteria, while exposure to low concentration may cause plasmoptysis.

2. Nutritional requirements

Microorganisms are often grouped according to their energy source and their source of carbon.

a. Energy source

1. Phototrophis: use radiant energy (light) as their primary energy source (photochemical reaction).

2. Chemotrophis: use the oxidation and reduction of chemical compounds as their primary energy source (chemical reaction).

b. Carbon source

Carbon is the structural backbone of the organic compounds that make up a living cell. Based on their source of carbon bacteria can be classified as autotrophs or heterotrophs.

1. **Autotrophis:** require only carbon dioxide as a carbon source. An autotroph can synthesize organic molecules from inorganic nutrients e.g. nitrates, nitrites, ammonium sulfate, ---.

2. **Heterotrophis:** require organic forms of carbon. A heterotroph cannot synthesize organic molecules from inorganic nutrients.

1. **Photoautotrophs:** use light as an energy source and carbon dioxide as their main carbon source.
- They include photosynthetic bacteria (green sulfur bacteria, purple sulfur bacteria, and cyanobacteria), algae, and green plants. Photoautotrophs transform carbon dioxide and water into carbohydrates and oxygen gas through photosynthesis

2. **Photoheterotrophs:** use light as an energy source but cannot convert carbon dioxide into energy. Instead they use organic compounds as a carbon source. They include the green nonsulfur bacteria and the purple nonsulfur bacteria.

3. **Chemolithoautotrophs:** use inorganic compounds such as hydrogen sulfide, sulfur, ammonia, nitrites, hydrogen gas, or iron as an energy source and carbon dioxide as their main carbon source.

4. **Chemoheterotrophs:** use organic compounds as both an energy source and a carbon source. Saprophytes live on dead organic matter while parasites get their nutrients from a living host. Most bacteria, and all protozoans, fungi, and animals are chemoorganoheterotrophs.

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c. Nitrogen source

Nitrogen is needed for the synthesis of such molecules as amino acids, DNA, RNA and ATP. Depending on the organism, nitrogen, nitrates, ammonia, or organic nitrogen compounds may be used as a nitrogen source.

d. Minerals

1. **Sulfur:** Sulfur is needed to synthesize sulfur-containing amino acids and certain vitamins. Depending on the organism, sulfates, hydrogen sulfide, or sulfur-containing amino acids may be used as a sulfur source.

2. **Phosphorus:** Phosphorus is needed to synthesize phospholipids, DNA, RNA, and ATP. Phosphate ions are the primary source of phosphorus.

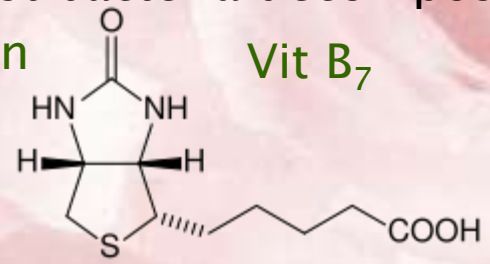
3. **Potassium, magnesium, and calcium:** These are required for certain enzymes to function as well as additional functions.

note: Nitrogen, Sulfur, Phosphorus are found in amino acids and proteins. (most bacteria decompose proteins)

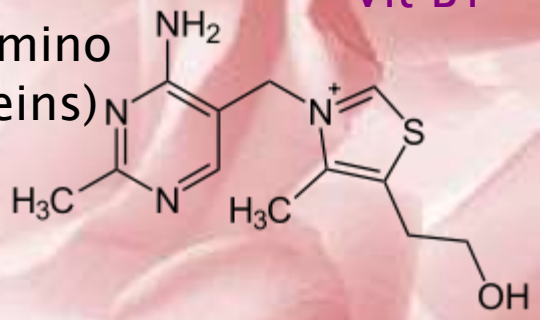
-S in thiamine and biotin

-Phosphate ions (PO_4^{3-})

Vit B_7



Vit B1



4. Iron: Iron is a part of certain enzymes.

5. Trace elements: Trace elements are elements required in very minute amounts, and like potassium, magnesium, calcium, and iron, they usually function as cofactors in enzyme reactions. They include sodium, zinc, copper, molybdenum, manganese, and cobalt ions. Cofactors usually function as electron donors or electron acceptors during enzyme reactions.

e. Water

f. Growth factors

Growth factors are organic compounds such as amino acids, purines, pyrimidines, and vitamins that a cell must have for growth but cannot synthesize itself. Organisms having complex nutritional requirements and needing many growth factors are said to be fastidious.

Biofilms

Microbial communities form slime or hydrogels

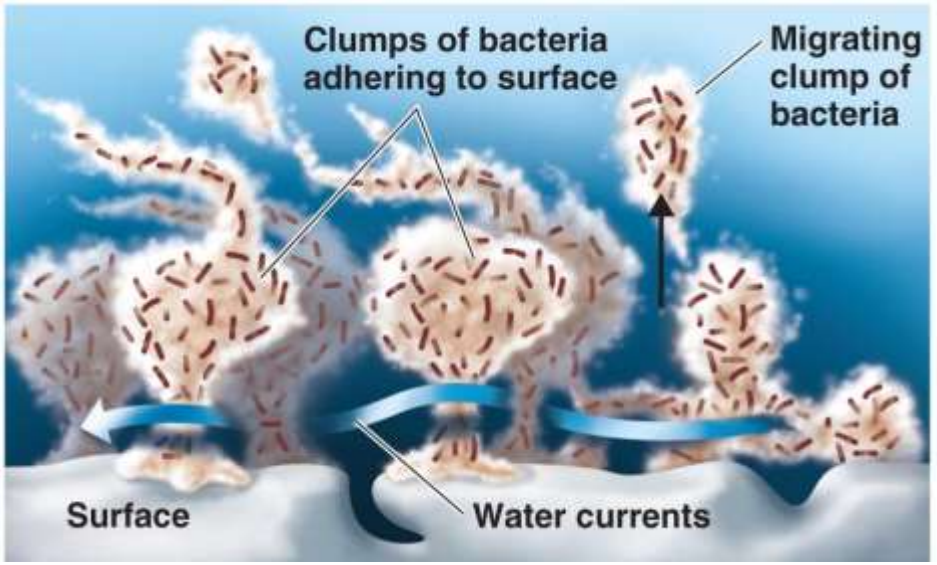
Starts via attachment of planctonic bacterium to surface structure.

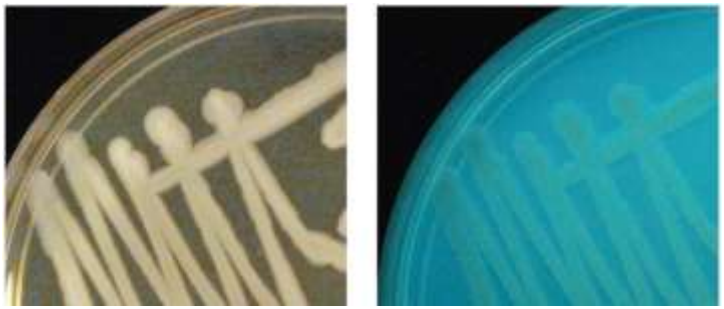
Bacteria communicate by chemicals via **quorum sensing**

Sheltered from harmful factors (disinfectants etc.)

Cause of most nosocomial infections

Clinical Focus: Delayed Bloodstream Infection Following Catheterization





Culture Media

- Culture medium: Nutrients prepared for microbial growth
- Have to be sterile (not contain living microbes)
- **Inoculum**: Microbes introduced into medium
- Culture: Microbes growing in/on culture medium
- Chemically defined media: Exact chemical composition is known (for research purposes only)
- **Complex media**: Extracts and digests of yeasts, meat, or plants, *e.g.:*
  - Nutrient broth
  - Nutrient agar
  - Blood agar

Agar

- Complex polysaccharide
- Used as solidifying agent for culture media in Petri plates, slants, and deeps
- Generally not metabolized by microbes
- Liquefies at 100°C
- Solidifies ~40°C

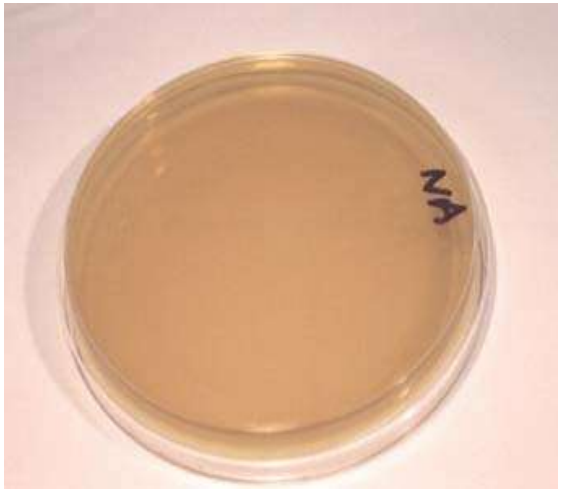
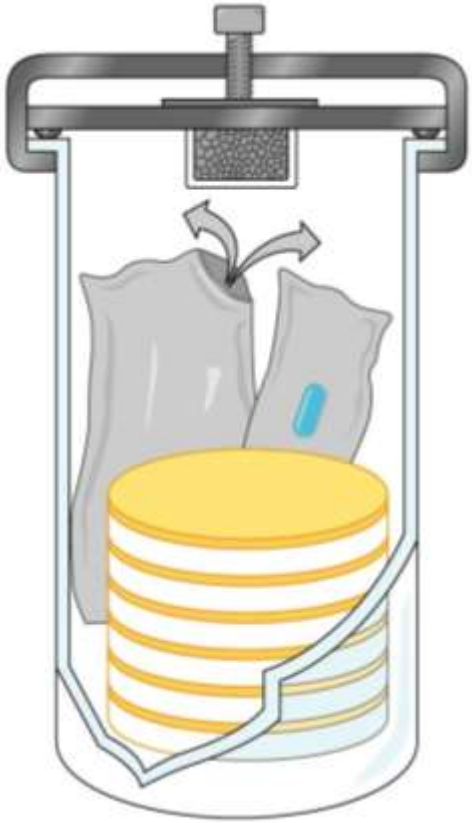


TABLE 6.4 **Composition of Nutrient Agar, a Complex Medium for the Growth of Heterotrophic Bacteria**

Constituent	Amount
Peptone (partially digested protein)	5.0 g
Beef extract	3.0 g
Sodium chloride	8.0 g
Agar	15.0 g
Water	1 liter

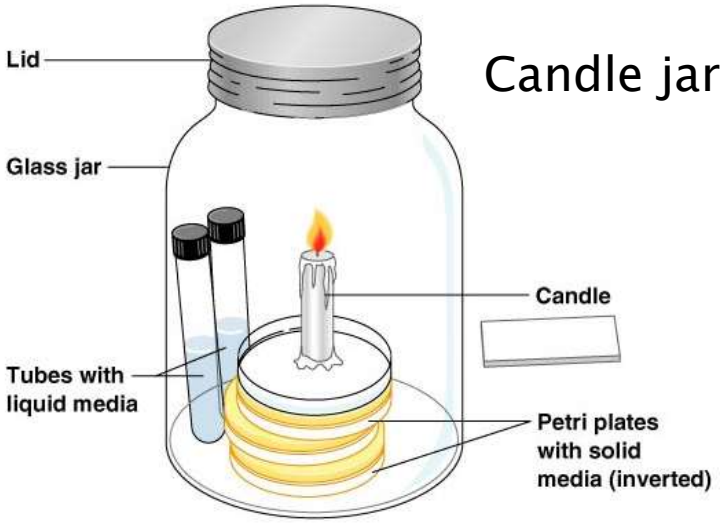
Anaerobic Culture Methods

- Use reducing media, containing chemicals (*e.g.*: thioglycollate) that combine with O_2
- Are heated shortly before use to drive off O_2
- Use anaerobic jar
- Novel method in clinical labs:
  Add **oxyrase** to growth media
  ⇒ OxyPlate (no need for anaerobic jar)

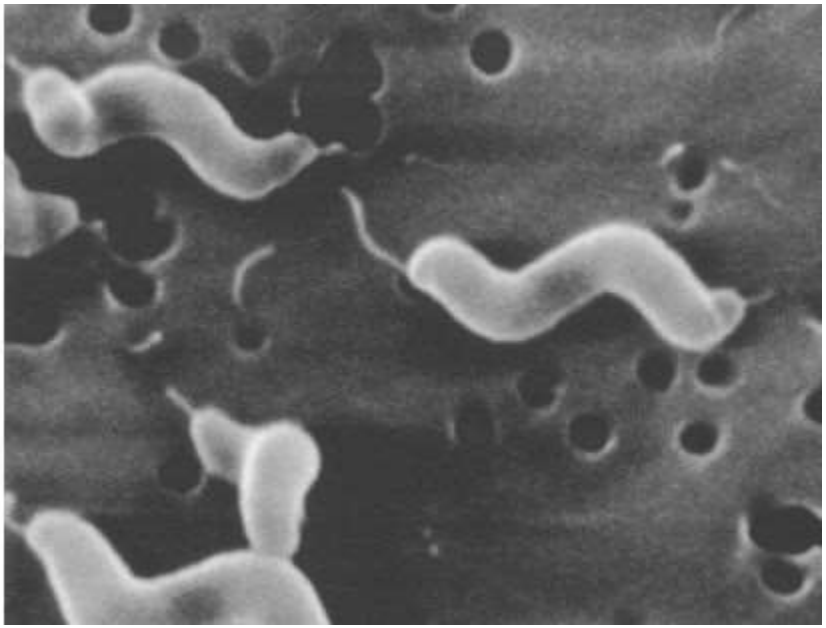


Capnophiles: Aerobic Bacteria Requiring High CO_2

- Low oxygen, high CO_2 conditions resemble those found in
  - intestinal tract
  - respiratory tract and
  - other body tissues where pathogens grow
- *E.g: Campylobacter jejuni*
- Use candle jar, CO_2-generator packets, or CO_2 incubators



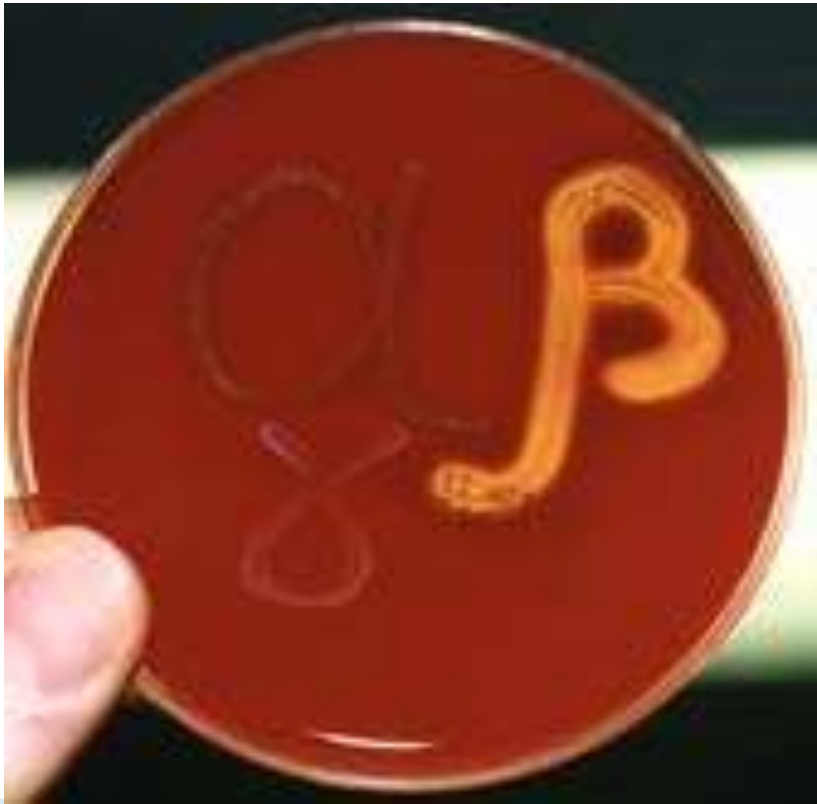
Candle jar



Selective Media and Differential Media

Selective medium: Additives suppress unwanted and encourage desired microbes – *e.g.* EMB, mannitol salt agar etc.

Differential medium: changed in recognizable manner by some bacteria ⇒ Make it easy to distinguish colonies of different microbes – *e.g.* α and β hemolysis on **blood agar**; MacConkey agar, EMB, mannitol salt agar etc.



Enrichment Media/Culture

- Encourages growth of desired microbe
- Example: *Assume soil sample contains a few phenol-degrading bacteria and thousands of other bacteria*
  - Inoculate phenol-containing culture medium with the soil and incubate
  - Transfer 1 ml to another flask of the phenol medium and incubate
  - Transfer 1 ml to another flask of the phenol medium and incubate
  - Only phenol-metabolizing bacteria will be growing

Pure Cultures

Contain only one species or strain.

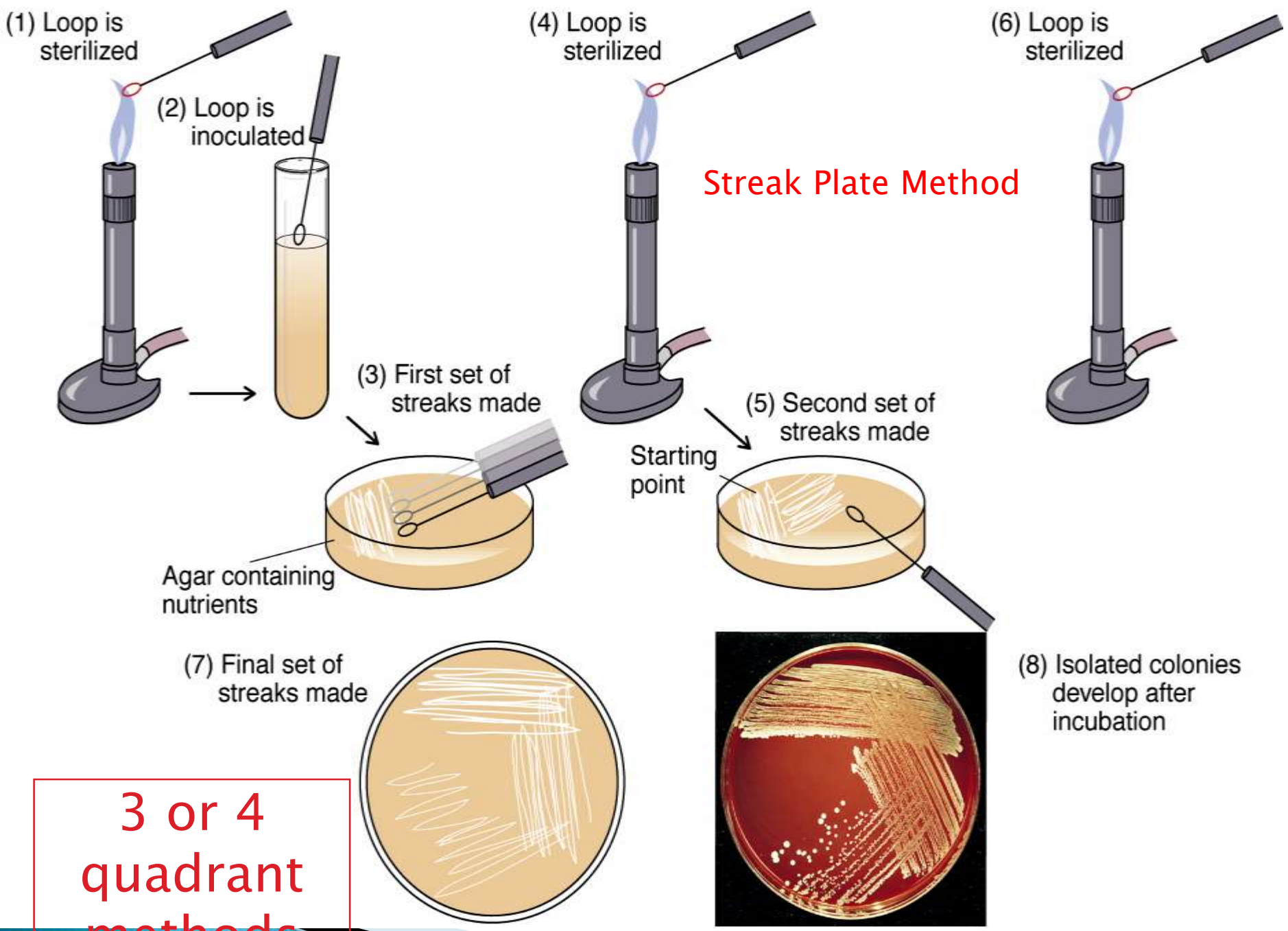
Most patient specimens and environmental samples contain several different kinds of bacteria

Streak-plate method is commonly used

Colony formation: A population of cells arising from a single cell or spore or from a group of attached cells (also referred to as **CFU**).

Only ~1% of all bacteria can be successfully cultured

Aseptic technique critical!



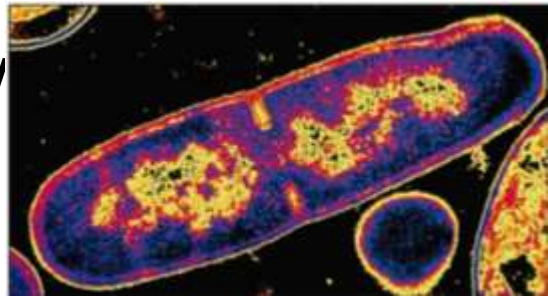
Streak Plate Method

3 or 4 quadrant methods

Preserving Bacterial Cultures

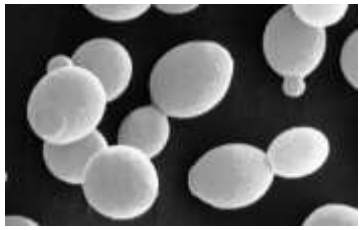
- **Deep-freezing**: Rapid cooling of pure culture in suspension liquid to –50°to –95°C. Good for several years.
- **Lyophilization** (freeze-drying): Frozen (–54° to –72°C) and dehydrated in a vacuum. Good for many years.

The Growth of Bacterial Cultures



Binary fission – exponential grow

Budding



Generation time – time required for cell to divide (also known as doubling time)

Ranges from 20 min *(E. coli)* to > 24h *(M. tuberculosis)*

Consider reproductive potential of *E. coli*

Fig 6.13

Numbers of Cells	Numbers Expressed as a Power of 2	Visual Representation of Numbers
1	2^0	●
2	2^1	●●
4	2^2	●●●●
8	2^3	●●●●●●●●
16	2^4	●●●●●●●●●●●●●●●●
32	2^5	●●●●●●●●●●●●●●●●●●●●●●●●●●●●●●●●

Generation Number	Number of Cells	Log_{10} of Number of Cells
0	$2^0 = 1$	0
5	$2^5 = 32$	1.51
10	$2^{10} = 1{,}024$	3.01
15	$2^{15} = 32{,}768$	4.52
16	$2^{16} = 65{,}536$	4.82
17	$2^{17} = 131{,}072$	5.12
18	$2^{18} = 262{,}144$	5.42
19	$2^{19} = 524{,}288$	5.72
20	$2^{20} = 1{,}048{,}576$	6.02

Antibiotics

By
Dr. Zainab Al-Hakeem
Immunologist in microbiology

Definitions:

Antibiotic: is a chemical substance produced by a microorganism that inhibits the growth of or kills other microorganisms.

An antimicrobial agent: is a chemical substance derived from a biological source or produced by chemical synthesis that kills or inhibits the growth of microorganisms.

Classification

Bactericidal drugs: kill the target bacterium or fungus and are more effective. These types of drugs are used to treat endocarditis, meningitis, osteomyelitis, and other invasive bacterial infections.

- Hosts with low immune systems should also be treated with bactericidal drugs.
- These drugs include β- Lactams, aminoglycosides, and fluoroquinolones.

Bacteriostatic: drugs inhibit growth and can be helpful cause they let the normal host defenses to destroy the microorganisms. These drugs are used to treat infections such as cystitis.

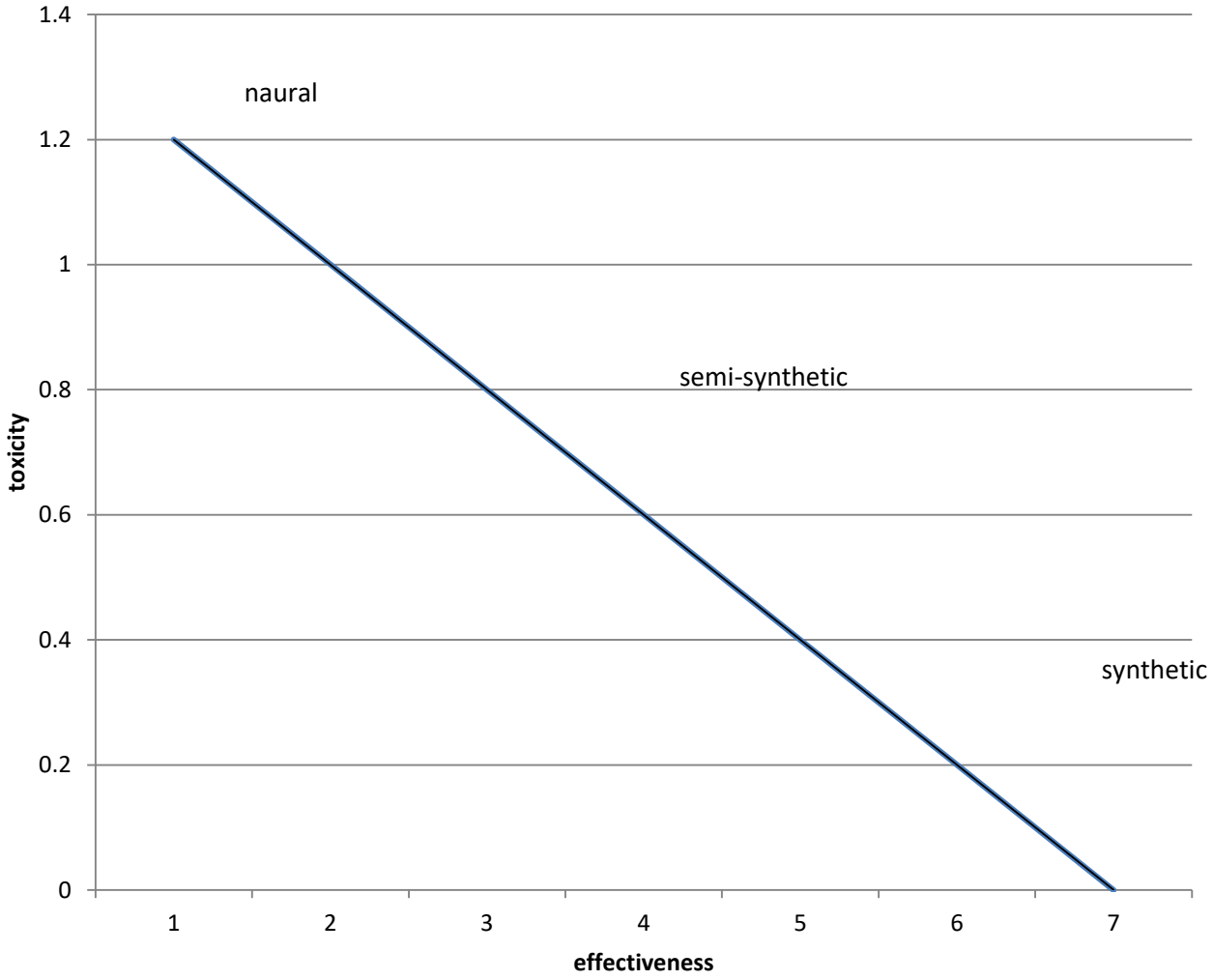
- These drugs include tetracycline, sulfonamides, clindamycin, and chloramphenicol. Some organisms require 2 agents for killing such as entercocci.

Source of antimicrobial agent:

❑Natural - mainly fungal sources. Organisms develop resistance faster to the natural antimicrobials because they have been pre-exposed to these compounds in nature. Natural antibiotics are often more toxic than synthetic antibiotics, e.g. **Benzylpenicillin** and **Gentamicin are** natural antibiotics.

❑Semi-synthetic - chemically-altered natural compound, they are drugs were developed to decrease toxicity and increase effectiveness, e.g. **Ampicillin**

❑Synthetic - chemically designed in the lab, they have an advantage that the bacteria are not exposed to the compounds until they are released. They are also designed to have even greater effectiveness and less toxicity, e.g. **Moxifloxacin.**



relationship of toxicity and effectiveness in natural, semi-synthetic and synthetic antibiotics

Role of antibiotic:

•The main role of antibiotic is to inhibit bacterial multiplication as they have a bacteriostatic effect which is occur at **MIC (minimum inhibitory concentration).**

➢MIC = lowest concentration of antibiotic that inhibits bacterial growth.

• To destroy or kill the bacterial population as Antibiotics have a bactericidal effect which occur at **MBC (Minimal Bactericidal Concentration).**

➢MBC is the lowest concentration of antibiotic that kills bacteria.

Classification of antibiotics and modes of action

A- Grouped by site of action, four functional groups cover most antibiotics:

1. Inhibitors of cell wall synthesis:

- Peptidoglycan forms the rigid, shape-maintaining layer of all medically important bacteria except mycoplasmas.
- Its structure is basically similar in Gram-positive and Gram-negative organisms, although there are important differences.
- It is a very important target for most antibiotic as it doesn't exist in eukaryotic cells.
- e. g. **Penicillins**, **cephalosporins** and other **b-lactam** agents, as well as **fosfomycin**, **cycloserine**, **bacitracin** and the glycopeptides, **vancomycin and teicoplanin,**
- All selectively inhibit different stages in the construction of the peptidoglycan.

2. Inhibitors of protein synthesis:

- Within bacterial cells, many ribosomes are engaged in protein synthesis during active growth, and a single strand of mRNA may interact with many ribosomes along its length to form polysome
- Many antibiotics interfere with the process of protein synthesis at any stage causing the production of immture protein chain.
- Therapeutically useful inhibitors of protein synthesis include many of the naturally occurring antibiotics, such as
- **chloramphenicol, tetracyclines, aminoglycosides, fusidic acid, macrolides, lincosamides and streptogramins**

3. Inhibitors of membrane function:

- Several antibiotics, known collectively as ionophores, interfere with cation transport in cell membranes.

- These include the topical antibiotic **gramicidin A,**

- and some agents used in veterinary medicine, such as the **macrotetralid emonensin** and the **valinomycin.**

- Naturally occurring antimicrobial peptides, such as the **cecropins**, **defensins**, all act in the same manner.

4.Inhibitors of nucleic acid synthesis:

Compounds that bind directly to the double helix and affect DNA synthesis

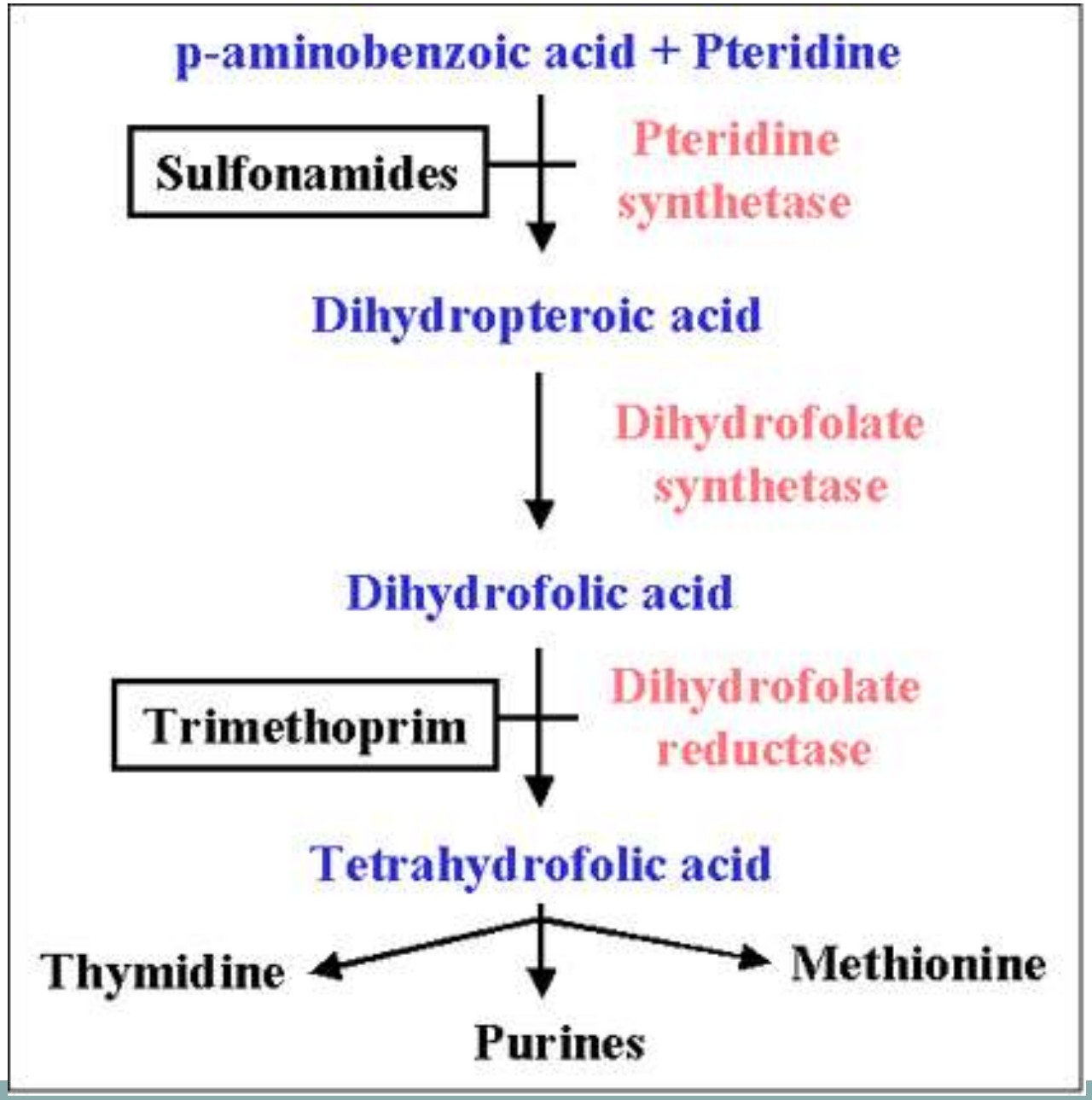
these compounds include antibacterial **quinolones**, **drifampicin (rifampin).**

B- grouped by their mode of action, we can recognize the following

1- Competition with a natural substrate for the active site of enzyme

A- Sulfonamides, sulfones (bacteriostatic): These antimicrobials are analogues of para-aminobenzoic acid and competitively inhibit formation of dihydropteric acid e. g. **Sulfonamides, sulfones**.

B- Antimicrobials bind to dihydrofolate reductase and inhibit formation of tetrahydrofolic acid e. g. **Trimethoprim**, **methotrexate, pyrimethamin**.


p-aminobenzoic acid + Pteridine
Sulfonamides
Pteridine synthetase
Dihydropteroic acid
Dihydrofolate synthetase
Dihydrofolic acid
Trimethoprim
Dihydrofolate reductase
Tetrahydrofolic acid
Thymidine
Purines
Methionine

2- Combination with an enzyme at a site sufficiently close to the active site as to interfere with its enzymatic function e.g. **vancomycin** and **bacteracin.**

3- Combination with non-enzymatic structural components, e.g. drugs which inhibits protein synthesis and damaging cytoplasmic membrane.

ANTIMICROBIAL DRUG RESISTANCE

A.PRINCIPLES AND DEFINITIONS:

1. Clinical Resistance

Clinical resistance to an antimicrobial agent occurs when the MIC of the drug for a particular strain of bacteria exceeds that which is capable of being achieved with safety in vivo. **Resistance to an antimicrobial can arise:**

I. By mutation in the gene that cods for either the target of the drug or the transport system in the membrane that controls the uptake of the drug.

II. By acquisition of extrachromosomal DNA (plasmid) carrying a resistance gene. It's important for the following reasons:

- It occur in many different species, especially G-ve rods.
- Plasmids mediated resistance to multiple drugs.
- Plasmids have a high transfer rate from one cell to another, usually by conjugation.
- Plasmids can replicate independently of bacterial chromosome.

2. Cross Resistance

- Cross resistance implies that a single mechanism confers resistance to multiple antimicrobial agents while multiple resistance implies that multiple mechanisms are involved.
- Cross resistance is commonly seen with closely related antimicrobial agents while multiple resistance is seen with unrelated antimicrobial agents.

B. MECHANISMS OF RESISTANCE:

1. Altered permeability of the antimicrobial agent

Altered permeability may be due to the inability of the antimicrobial agent to enter the bacterial cell or alternatively to the active export of the agent from the cell.

2. Inactivation of the antimicrobial agent

Resistance is often the result of the production of an enzyme that is capable of inactivating the antimicrobial agent.

3. Altered target site

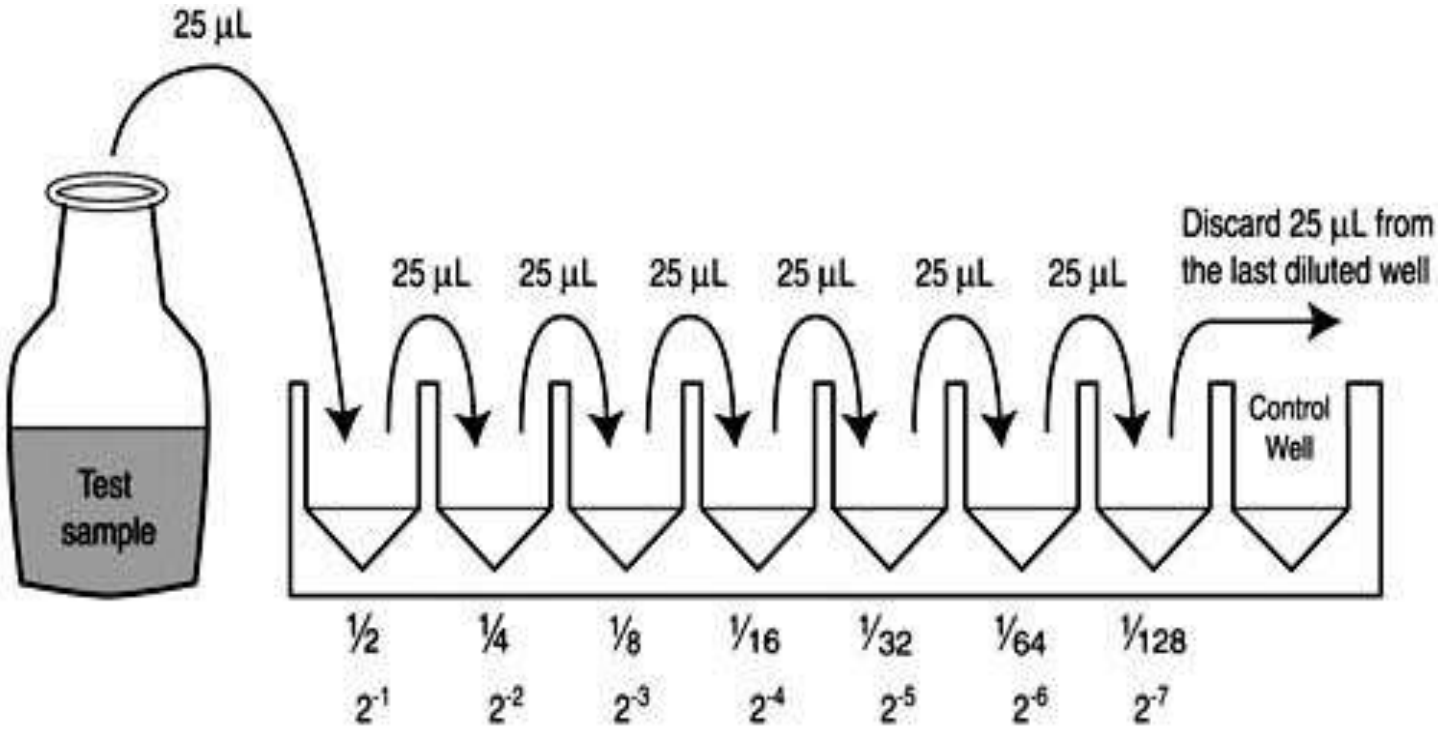
Resistance can arise due to alteration of the target site for the antimicrobial agent.

4. Replacement of a sensitive pathway

Resistance can result from the acquisition of a new enzyme to replace the sensitive one.

Antibiotic Sensitivity Testing Methods

Dilution method: preparing two-fold dilutions of antibiotics (eg, 1, 2, 4, 8, and 16 µg/mL) in a liquid growth medium inoculated with a standardized bacterial suspension of $1–5\times10^5$CFU/mL. Following overnight incubation at 35°C, the tubes were examined for visible bacterial growth it is useful in endocarditis and in slow growing bacteria like tubercle bacilli

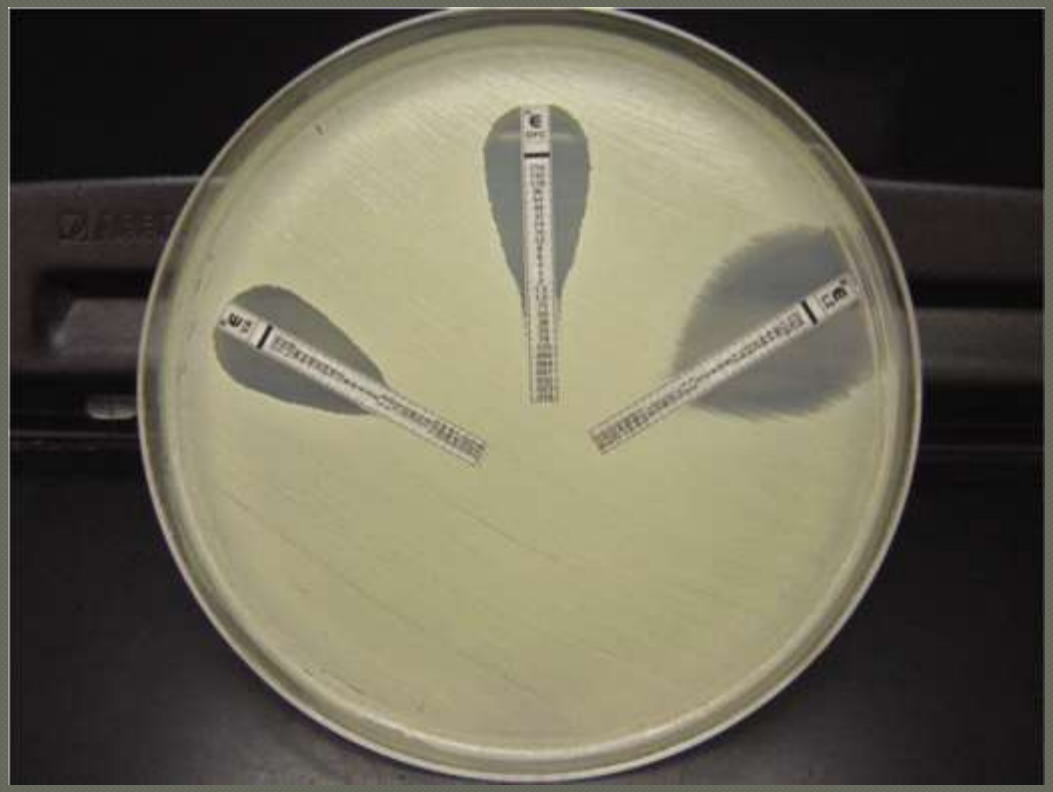


Disk diffusion method:

- The test is performed by applying bacterial inoculums of approximately 1–2×10^8CFU/mL to the surface of a large (150 mm diameter) agar plate
- a bacterial isolate is tested for resistance to each of twelve different antibiotics.
- The clear zones around each disc are the zones of inhibition that indicate the extent of the test organism's inability to survive in the presence of the test antibiotic.

•E-test: it utilizes a plastic test strip impregnated with a gradually decreasing concentration of a particular antibiotic.

•The strip also displays a numerical scale that corresponds to the antibiotic concentration contained therein.



•Automated antimicrobial susceptibility testing system: Use of instrumentation can standardize the reading of end points and often produce susceptibility test results in a shorter period than manual readings because sensitive optical detection systems allow detection of subtle changes in bacterial growth.

•Gynotypic method: Some of the most common molecular techniques utilized for antimicrobial resistance detection are PCR and DNA hybridization.

Laboratory use of antibiotic:

1. They me be incorporated as selective agents in culture mediae.g. penicillin could be used in isolation of *Hemophilusinfluenza*
2. They are used to control the bacterial contamination in tissue culture used for virus isolation e.g. **penicillin streptomycin**, **nystatin**,ect….
3. Epidemiological value.

Antibiotic assay in body fluids:

- It is done by making serial dilution of specimen of body fluid and inoculating standard suspensions of bacteria of known minimum inhibitory concentration (MIC).
- It is useful in verifying adequate drug concentration in blood and other body fluid.
- It also guid against excessive blood levels of toxic drugs.

Antimicrobial chemoprophylaxis:

❖it is of great help to prevent the infection.

❖Soon after the entry and establishment of microorganism but before the development of symptoms antimicrobial chemoprophylaxisis useful, e.g. in a compound fracture.

❖Other situations where antimicrobial chemoprophylaxis required are:

A- Heart diseases like valve abnormalities It can be prevented if proper drug is administrated during the period of bacteremia e.g. **penicillin**, **erythromycin** for streptococcal and **penicillin** **gentamycinin** cases of enterococcal organism.

B- Antibiotics also may be prescribed to prevent infections in people with weakened immune systems, **such as people with AIDS** or people who are having **chemotherapy treatments for cancer.**

C- Healthy people with strong immune systems if they are traveling to parts of the world where they are likely to get an infection that causes diarrhea, for example.

D- In respiratory tract diseases and in UTI. For the first the most common organism involved is Pneumonococci, *Hemophilus influenza*, *pseudomonas*, *staphelococcus*, *proteus*, ect. Chemoprophylaxis consists of **ampicillin**, **tatracyclin**, **trimethoprim-sulfamethoxazole.**

Introduction: some important definitions (lec1 cout.)

By
Dr. Zainab M Al Hakeem
Immunologesit in microbiology

•**Infection**

The entry, establishment, & multiplication of pathogenic organisms within a host.

•**Primary infection**

An initial infection in a previously healthy individual that is later complicated by an additional (secondary) infection.

•**Secondary infection**

An infection that compounds an preexisting one.

•**Acute infections**

Characterized by rapid onset & short duration.

•**Chronic infections**

Any process or disease that persists over a long duration.

•Localized infection

Occurs when a microbe enters a specific tissue, infects it, & remains confined there.

•Systemic infection

Infections that invade many compartments & organs via the circulation. Occurring throughout the body.

•Focal infection

Occurs when an infectious agent breaks loose from a localized infection & is carried by the circulation to other tissues.

•Incubation period

The period from the initial contact with an infectious agent to the appearance of the first symptoms.

•Prodromal stage

A short period of mild symptoms occurring at the end of the period of incubation. Indicates the onset of disease.

•Period of invasion

The period during a clinical infection when the infectious agent multiplies at high levels, exhibits its greatest toxicity, & becomes well established in the target tissues.

•Convalescent period

Recovery; the period between the end of the disease & the complete restoration of health in a patient.

•Mixed infection (Also known as synergistic infection)

Occurs when several different pathogens interact simutaneously to produce an infection.

•Portal of entry

Route of entry for an infectious agent; typically a cutaneous or membranous route.

•**Pathogen**

Any agent (usually a virus, bacterium, fungus, protozoan, or helminth) that cause a disease.

•**Pathogenicity**

The capacity of microbes to cause a disease or infection.

•**True pathogens (primary pathogens)**

A microbe capable of causing infection & disease in healthy persons with normal immune defenses.

•**Opportunistic pathogens**

Cause disease when the host's defenses are compromised or when they become established in a part of the body that is natural to them.

•**Virulence**

In infection, the relative capacity of a pathogen to invade and harm host cells.

•**Virulence factor**

A microbe's structures or capabilities that allow it to establish itself in a host & cause damage.

•**Pathologic**

Capable of inducing physical damage to the host.

•**Infectious disease**

The state of damage or toxicity in the body caused by an infectious agent.

•**Toxigenicity**

The tendency of a pathogen to produce toxins. Power to produce toxins.

•**Toxinoses**

Disease whose adverse effects are primarily due to production & release of toxins.

•**Toximias**

Condition in which a toxin (microbial or otherwise) is spread throughout the bloodstream.

•**Intoxications (botulism)**

Caused by ingestion of toxins.

Bacterial Pathogenesis

- A pathogen is a microorganism (or virus) that is able to produce disease.
- Pathogenicity is the ability of a microorganism to cause disease in another organism, namely the host for the pathogen.
- As implied above, pathogenicity may be a manifestation of a host-parasite interaction.

- In humans, some of the normal bacterial flora (e.g. *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Haemophilus influenzae)* are potential pathogens that live in a commensal or parasitic relationship without producing disease.
- They do not cause disease in their host unless they have an opportunity brought on by some compromise or weakness in the host's anatomical barriers, tissue resistance or immunity.
- Furthermore, the bacteria are in a position to be transmitted from one host to another, giving them additional opportunities to colonize or infect.

- There are some pathogens that do not associate with their host except in the case of disease.
- These bacteria may be thought of as obligate pathogens, even though some may rarely occur as normal flora, in asymptomatic or recovered carriers, or in some form where they cannot be eliminated by the host.

Opportunistic Pathogens

- Bacteria which cause a disease in a compromised host which typically would not occur in a healthy (noncompromised) host are acting as opportunistic pathogens.
- A member of the normal flora can such as *Staphylococcus aureus* or *E. coli* can cause an opportunistic infection, but so can an environmental organism such as *Pseudomonas aeruginosa*.
- When a member of the normal flora causes an infectious disease, it sometimes referred to as an endogenous bacterial disease, referring to a disease brought on by bacteria 'from within'.
- Classic opportunistic infections in humans are dental caries and periodontal disease caused by normal flora of the oral cavity.

Infection

- The normal flora, as well as any "contaminating" bacteria from the environment, are all found on the body surfaces of the animal; the blood and internal tissues are sterile.
- If a bacterium, whether or not a component of the normal flora, breaches one of these surfaces, an infection is said to have occurred.
- Infection does not necessarily lead to infectious disease. In fact, infection probably rarely leads to infectious disease.
- Some bacteria rarely cause disease if they do infect; some bacteria will usually cause disease if they infect.
- But other factors, such as the route of entry, the number of infectious bacteria, and (most importantly) the status of the host defenses, play a role in determining the outcome of infection.

Determinants of Virulence

❖Pathogenic bacteria are able to produce disease because they possess certain structural or biochemical or genetic traits that render them pathogenic or virulent.

❖(The term virulence is best interpreted as referring to the degree of pathogenicity.)

❖The sums of the characteristics that allow a given bacterium to produce disease are the pathogen's determinants of virulence.

❖Some pathogens may rely on a single determinant of virulence, such as toxin production, to cause damage to their host.

❖Thus, bacteria such as *Clostridium tetani* and *Corynebacterium diphtheriae*, which have hardly any invasive characteristics, are able to produce disease, the symptoms of which depend on a single genetic trait in the bacteria: the ability to produce a toxin.

❖Other pathogens, such as *Staphylococcus aureus*, *Streptococcus pyogenes* and *Pseudomonas aeruginosa*, maintain a large No. of virulence determinants and consequently are able to produce a more complecated range of diseases that affect different tissues in their host.

HOME WORK

Write a short definition(s) about the following terms

- microbiome
- microbiosis
- microbion

Shigella:

- **Species of this genus are Gram-negative, rod-shaped, and non-lactose fermenting (with exception to *S. sonnei*). They are also non-capsulated, non-motile due to the lack of flagella and make ATP (an energy molecule) by aerobic respiration if oxygen is present, but is also capable of switching to fermentation - facultative anaerobe.**

- **Optimal growth temperature 37°C pH 7.4, isolated from mixed culture by using selective media such as DCA agar - Deoxycholate Citrate Agar to form colorless colonies.**
- **Shigella species can ferment carbohydrates with the production of acid, and grow in translucent white colonies.**
- **There are four species of Shigella: *S. boydii, S. dysenteriae, S. flexneri, and S. sonnei.***
- **On nutrient agar, after overnight growth colonies are small, 2 mm in diameter, circular, convex, smooth and translucent.**
- **On broth culture there is uniform growth with mild turbidity after 12-24 hrs of incubation.**

Biochemical reactions:

- **Shigella species are negative for motility.**
- **They do not ferment lactose (However, *S. sonnei* can ferment lactose).**
- **They typically do not produce gas from carbohydrates (with the exception of certain strains of *S. flexneri*) and tend to be overall biochemically inert.**
- **Shigella should also be urea hydrolysis negative**

- When inoculated to a triple sugar iron (TSI) slant, they react as follows: K/A, gas -, H2S -. Indole reactions are mixed, positive and negative, with the exception of *S. sonnei*, which is always indole negative.

- Growth on Hektoen enteric agar will produce bluish-green colonies for Shigella and bluish-green colonies with black centers for Salmonella.

Antigenic structure:

For Shigella, only the O-antigen classification system is used, as they lack H and K antigens. The O-antigen represents the polysaccharide chain (O-polysaccharide, OPS) of the lipopolysaccharide (LPS), which is usually built up of repeating units containing two to eight sugar residues and often also non-sugar substituents (e.g., amino acids, pyruvic acid acetals, lactic acid ethers, phosphate, O-acetyl groups, etc.).

Toxins:

There are many terms that microbiologists use to describe Shiga toxin and differentiate between different forms of it. Many of these terms are used interchangeably.

1. Shiga toxin (Stx) - true Shiga toxin is produced by *Shigella dysenteriae*.

2. Shiga-like toxin 1 and 2 (SLT-1 and 2 or Stx-1 and 2) - the Shiga toxins produced by some E. coli strains. SLT-1 differs from Stx by only 1 amino acid. SLT-2 shares 56% sequence homology with SLT-1.

3. Cytotoxins - an archaic denotation for Stx, used in a broad sense.

4. Verocytotoxins/verotoxins - a seldom used term for Stx, from the hypersensitivity of Vero cells to Stx.

Classification:

The genus is divided into four serogroups with multiple serotypes: A (*S dysenteriae*, 12 serotypes); B (*S flexneri*, 6 serotypes); C (*S boydii*, 18 serotypes); and D (*S sonnei*, 1 serotype).

Pathogenicity:

Shigella causes Shigellosis an infection that typically occurs via ingestion (fecal–oral contamination); depending on age and condition of the host, less than 100 bacterial cells can be enough to cause an infection. Shigella causes dysentery that results in the destruction of the epithelial cells of the intestinal mucosa in the cecum and rectum. Some strains produce enterotoxin and shiga toxin, similar to the verotoxin of *E. coli* O157:H7, and other verotoxin-producing *Escherichia coli*. Both shiga toxin and verotoxin are associated with causing hemolytic uremic syndrome.

Enterotoxic and / or Cytotoxic diarrhoeal prodrome, Followed by

- **Cytokine-mediated inflammation of the colon, and necrosis of the colonic epithelium.**
- **The rectosigmoidal lesions of shigellosis resemble those of ulcerative colitis**

invasion of Shigella into the colonic epithelium and the lamina propria initiates inflammatory cascade.

- Colitis and ulceration of the mucosa result in bloody, mucoid stools, and/or febrile diarrhoea.

Shigella colonise large intestine

• Multiply within12 hrs

• Infection is initiated at the microfold (M) cells of Peyer's patches.

• Lyse phagocytic vacuole, replicate in cytoplasm. Phagocytosed by resident macrophages in the subepithelial space,.

•Infected macrophage releases the inflammatory cytokine IL-1, which elicits infiltration of PMN.

•Affected areas edematous, with capillary congestion, focal hemorrhage, crypt hyperplasia,

•mononuclear and PMN cell infiltration

The infection lasts five to seven days, but children, the elderly, and individuals who are immunosuppressed are more prone to severe cases of shigellosis. Symptoms can take about a week to manifest itself, and the bacteria stays in the host for about a week or two after the illness has surpassed, and thus is still transferable.

Shigella invades the host through the M-cells in the gut epithelia of the small intestine, as they cannot enter directly through the epithelial cells. Using a Type III secretion system acting as a biological syringe, the bacterium injects certain protein into cells, triggering bacterial invasion and the subsequent lysis of vacuolar membranes using proteins. It uses a mechanism for its motility by which is a protein triggers actin polymerization in the host cell in a "rocket" propulsion fashion for cell-to-cell spread. The most common symptoms are diarrhea, fever, nausea, vomiting and stomach cramps.

The stool may contain blood, mucus, or pus. In rare cases, young children may have seizures. Symptoms can take as long as a week to show up, but most often begin two to four days after ingestion. Symptoms usually last for several days, but can last for weeks. Shigella is implicated as one of the pathogenic causes of reactive arthritis worldwide.

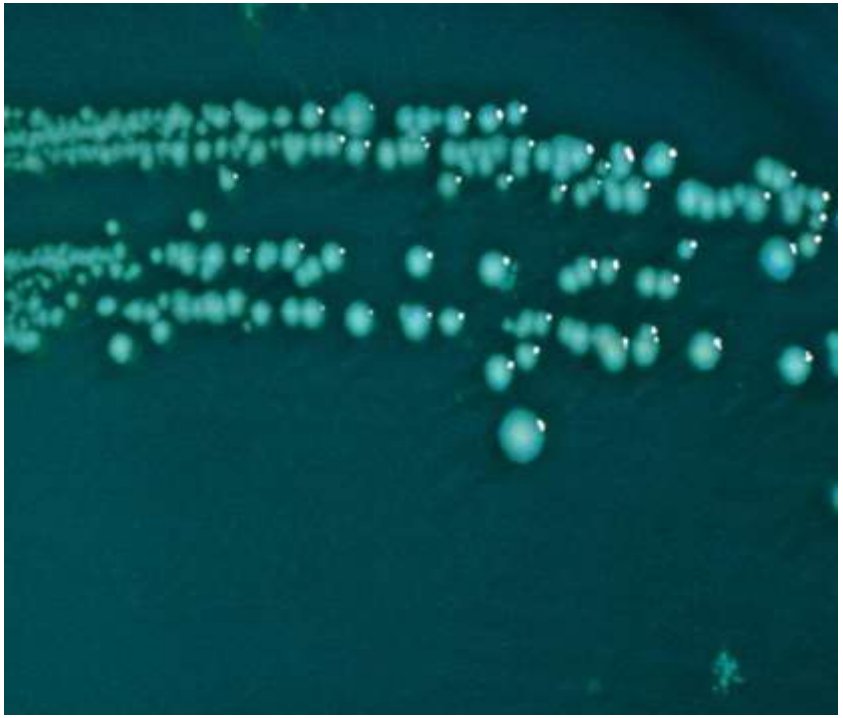
Each of the Shigella genomes includes a virulence plasmid that encodes conserved primary virulence determinants. The Shigella chromosomes share most of their genes with those of E. coli K12 strain MG1655.

Diagnosis:

laboratory tests must be performed by rectal or fecal swab tests. Isolation of shigellae in the clinical laboratory typically involves an initial streaking for isolation on differential/selective media with aerobic incubation to inhibit the growth of the anaerobic normal flora. Commonly used primary isolation media include

1. MacConkey, it is a differential media that result in G-ve bacteria.

2. Hektoen Enteric Agar: the transparent colonies, indicating the absence of carbohydrate fermentation. The lack of black colonies indicates no H2S production. All species of Shigella have this appearance on Hektoen enteric agar.



3. **Salmonella-Shigella (SS) Agar. These media contain bile salts to inhibit the growth of other Gram-negative bacteria and all +ve bacteria (selective) and pH indicators to differentiate lactose fermenters (Coliforms) from non-lactose fermenters such as shigellae.**

4. **Indole reactions are sometimes positive and sometimes negative, with the exception of *S. sonnei* which is always negative.**

SS agar for Salmonella & Shigella

Salmonella..Transparent colonies+H_2s.. Ohers fecal flora will be inhibited to 98%

Salmonella

Shigella

Treatment:

1. **Although severe dehydration is uncommon in shigellosis, the first consideration in treating any diarrheal disease is correction of abnormalities that result from isotonic dehydration, metabolic acidosis, and significant potassium loss.**

2. Absorbable drugs such as ampicillin (2 g/day for 5 days) are likely to be effective when the isolate is sensitive.

3. Trimethoprim (8 mg/kg/day) and sulfamethoxazole (40 mg/kg/day) will eradicate sensitive organisms quickly from the intestine (not give to children under 17 years).

Gram-negative bacteria

Proteus:

Morphology: A genus of gram-negative, facultative anaerobic, rod-shaped, non-sporulating bacteria, that occurs in the intestines of humans and a wide variety of animals, as well as in manure, soil, and polluted waters. Its species are pathogenic only when they are outside of their natural sites, causing urinary tract infections and are also considered secondary invaders, causing septic lesions at other sites of the body.

The most important Spp. Are:

Proteus mirabilis

Proteus vulgaris

- ***Proteus mirabilis* major agent in human infection of urinary tract**
- **They are abundant production of urease in which:**
- **Urease splits urea (CO(NH2)2) into carbon dioxide (CO2) and ammonia stones (renal stones; renal calculus) which can be extremely painful**
- **Wound infections, pneumonia, septicemia**

Found in soil, water, sewage, decomposing matter and human intestinal tract

- **Hypermotile with cultures demonstrating a swarming phenomenon on agar media at 37°c after 12-18 hours of incubation, swarming may be due to progressive surface growth spreading from the edge of parent colony. It can be suppressed by:**

1. **Chloral hydrate.**
2. **Sodium azide (1:500).**
3. **Alcohol (5%-6%).**
4. **Sulfonamide.**
5. **Boric acid (1:1000).**

- **Hydrogen sulfide (H2S) production is positive.**
- **O, H, K antigens: grouping on basis of these antigens for epidemiologic studies have not been correlative**
- **Share antigens with the intracellular pathogen Rickettsia, as well as cross reactivity with typhus patients because of common antigenic structure with o-Ag of salmonella.**
- **Treatment: ampicillin and cephalosporins, but resistant strains can develop.**

Table 1: biochemical reactions of *P. mirabilis* and *P. vulgaris*

	Glucose	L	Urease	MR	VP	H2S	Indole	Citrte
P. mirabilis	+	–	+	+	–	+	–	±
P. vulgaris	+	–	+	+	–	+	+	+